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THE JOURNAL OF HYGIENE

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CONTENTS

No. 1 (JULY)

	PAGE
WILSON, WM. H. The Diet Factor in Pellagra	1
WORDLEY, E. A New Method for the Isolation of Organisms from Faeces and Sputum, with some observations on Haemolytic Streptococci in Faeces obtained by this Method	60
SAVAGE, WILLIAM G. Studies upon the Toxicity of Putrid Food	69
TWORT, F. W. and TWORT, D. N. An Investigation on Influenza	85

No. 2 (OCTOBER)

PETRIE, G. F. The Toxigenic Features of Strains of the Diphtheria Bacillus isolated from Horses and from a Mule	99
TOPLEY, W. W. C. The Spread of Bacterial Infection. The Potential Infectivity of a surviving Mouse-population, and their Resistance to Subsequent Epidemics of the same Disease. (With 2 Charts)	103
GARDNER, A. DUNCAN and WALKER, E. W. AINLEY. An Inquiry into the Nature of the Serological Differences exhibited by different Cultures of a Bacterial Species (<i>B. typhosus</i>). (With 5 Charts)	110
FREEAR, K., MATTICK, A. T. R. and WILLIAMS, R. STENHOUSE. A Study of the Bacteriological Examination of Grade "A" (Cert.) Milk	125
HARRIES, E. H. R. and WILLIAMS, R. STENHOUSE. Bovine Tuberculosis: the Etiological Support of Family History	132
DUDGEON, LEONARD S., WORDLEY, ERIC, and BAWTREE, F. On <i>Bacillus coli</i> Infections of the Urinary Tract, especially in Relation to Haemolytic Organisms	137
MATTICK, A. T. R. The Sterilization of Empty Milk Churns by Steam under Pressure	165
BUXTON, J. B. and ALLEN, H. R. Air Carriage of Pathogenic and other Organisms	173
GLENNY, A. T. and SÜDMERSEN, H. J. Notes on the Production of Immunity to Diphtheria Toxin. (With 15 Charts)	176

No. 3 (NOVEMBER)

	PAGE
TOPLEY, W. W. C., BARNARD, J. E., and WILSON, G. S. A New Method of obtaining Cultures from Single Bacterial Cells. (With Plate I)	221
TOPLEY, W. W. C., WEIR, H. B., and WILSON, G. S. The Interrelationships between the various members of the <i>B. enteritidis</i> — <i>B. paratyphosus</i> B Group of Bacteria. (With 3 Charts)	227
YOUNG, MATTHEW. A Preliminary study of the Epidemiology of Rheumatic Fever	248
BRUCE, MAJOR-GENERAL SIR DAVID. Trench Fever. Final Report of the War Office Trench Fever Investigation Committee	258
GALE, A. M. Some Points in the Epidemiology of an Outbreak of Cerebro-spinal Fever in Hong-Kong, 1918. (With 3 Charts, 2 Maps and 1 Figure)	289

No. 4 (DECEMBER)

KHALED, Z. A Comparative Study of Bovine Abortion and Undulant Fever, from the Bacteriological point of view. (With 2 Charts)	319
SCHÜTZE, H. The Permanence of the Serological Paratyphoid B Types, with Observations on the Non-specificity of Agglutination with "Rough" Variants. (With 1 Figure)	330
HOLST, PETER M. Studies on the Effects of Tuberculin. (With 2 Text-figures)	342
WORDLEY, E. Further Results on the Isolation of Organisms from Faeces by a New Method	360
EIJKMAN, C. Pulmonary Tuberculosis and the "Curvature of Van Pesch." (With 2 Text-figures)	363
HEIBERG, DR POVL. The Diet in the Different Grades of Society in Denmark	366
FREEAR, K., MATTICK, A. T. R. and WILLIAMS, R. STENHOUSE. The Keeping Qualities of Grade A (Certified) Milk. (With 1 Chart)	371
MITCHELL, J. ALEXANDER. Plague in South Africa: Perpetuation and Spread of Infection by Wild Rodents	377
BIGGER, JOSEPH W. The Reliability of the Wassermann Test as performed by different Pathologists	383
INDEX OF AUTHORS	390
INDEX OF SUBJECTS	391

THE DIET FACTOR IN PELLAGRA.

By WM. H. WILSON, M.A., M.D. (Oxon).

(*Professor of Physiology. School of Medicine, Cairo.*)

(With 22 Tables and 3 Charts.)

CONTENTS.

	PAGE
HISTORICAL SUMMARY	2
Summary of writer's conclusions	7
INTRODUCTION. METHOD OF INVESTIGATION ADOPTED. (With Tables I and II) .	8
Generally accepted facts related to the epidemiology of pellagra .	8
Discussion regarding K. Thomas' estimate of the biological value of protein from different sources	9
Individual variations in the minimum protein requirement, and the minimum biological value required by adult men	9
Table I. Estimate of individual protein requirement from Chittenden's observations	13
Table II. Biological value of the total metabolised protein in the same observations	14
PART I. ANALYSIS OF VARIOUS DIETS WITH THE INCIDENCE OF PELLAGRA AMONG THE AFFECTED COMMUNITIES. (With Tables III-XX and Charts I-III) .	15
1. Armenian refugee epidemic	15
Diet 7. Table III. Original diet	16
Table IV. Age distribution of inmates of refugee camp	17
Table V. Nutritive value of rations distributed according to age requirements	18
Table VI. Distribution of pellagra by age and sex	19
Diet 7a. Improved diet (1916)	19
Chart I. The same shown graphically	20
Diet 8. Table VII. Improved diet (1917)	21
Diet 9. Table VIII. Anti-pellagrous diet	21
Chart II. Relation of the biological value of protein in diets 7, 7a, 8 and 9 to pellagra incidence	22
2. Rankin Farm Experiment	23
Diet 10. Table IX. Diet used	23
3. Incidence of pellagra among Italian peasants	24
Diet 11. Table X. Eight months. XI. Four months of year	24
4. Individual cases among Egyptian fellahin. Diets 1-6	26
5. Incidence of pellagra among Turkish prisoners of war in Egypt	28
Diet 12. Table XII. No labour	29
Diet 13. Table XIII. Labour	29
6. Incidence of pellagra among convicts in Egypt	30
Diet 15. Table XIV. Hard labour	31
Diet 14. Table XV. Light and moderate labour	31
Statistics of pellagra at the Tura Convict Establishment	31
Table XVI. Relation of pellagra incidence to class of labour	32
Diet 16. Table XVII. Special diet for pellagrous prisoners	33

	PAGE
7. Intra-mural cases of pellagra. Egyptian Asylums	34
Diet 17. Table XVIII. Ordinary diet of inmates	34
8. A diet of medium value used successfully in the treatment of pellagra .	36
Diet 18. Table XIX. Ordinary diet. Kasr-el-Aini Hospital	36
9. Non-pellagrous diets	37
Diet 19, 20. Responsible for epidemic dropsy	37
Diet 21, 22. Japanese prisoners	37
Diet 23. Mean of six Indian gaol dietaries	38
Diet 24, 25. English sewing girl	38
Diet 26, 27. Scotch prison dietaries	38
Diet 28. English professional man	39
Résumé of deductions from Table XX	39
Description of Chart III	39
Table XX. General table of diets described in Part I	40
Chart III. Relation of different diet factors (as shown in Table XX) to pellagra	41
PART II. DISCUSSION OF VARIOUS FACTORS HAVING A POSSIBLE RELATIONSHIP TO THE ETIOLOGY OF PELLAGRA. (With Tables XXI and XXII)	42
Accessory food substances	42
Fats	43
Table XXI. Diets of low fat value	44
Lipoids	45
Protein	46
The value of maize	47
Significance of indicanuria	48
Table XXII. Degree of indicanuria in groups of healthy men, quiescent pellagra and active pellagra cases. (Note)	50
A-chlorhydria	51
Individual variations	53
Influence of work	54
General remarks	54
Conclusions	55
References	57

HISTORICAL SUMMARY.

THIS subject has been dealt with by so many investigators and, within recent years in the United States, in such minute detail that it would seem surprising that no definite conclusion has yet been reached as to the cause of the disease or the actual part played by the diet in bringing about the condition.

For an historical account of Pellagra and details of the various toxic and other theories of its etiology the text-books of A. Marie (1910), Roberts (1912) and Wood (1912) may be consulted; a brief epitome of work which has a bearing on the relation of pellagra to food deficiency must however be given.

As early as 1735 Casal of Oviedo in Spain who appears to have been the first to describe the disease connected it with poverty and bad nourishment.

Roberts quotes Townsend (1787) who visited Oviedo in 1787 as stating that "the people among whom the disease originates eat little flesh... their diet is Indian corn, with beans, peas, chestnuts, apples, pears, melons and cucumbers."

Roussel (1866) found that pellagra can be cured by good food and that without this remedies were useless.

Since the year 1898 when Sandwith may be said to have brought pellagra to the notice of English clinicians and especially since 1907 at about which date, according to Wood (1909), the disease first appears to have been seen to any considerable extent in the United States, the nature of the dietetic defect and the possibility of infective or other causes have given rise to a very copious literature of which reference can only be made to a small part, in particular to those investigations bearing on a deficiency in the quantity or quality of the protein as the causative factor.

As to whether maize as such is the cause of the disease, a theory originally propounded by Strambio in the eighteenth century (1786) and revived in Italy by Lombroso, is ably and fully discussed by Sambon (1910) who shows conclusively that pellagra may occur in persons who have never consumed any maize.

An experiment carried out at the State Asylum at Peoria, Illinois (1912), is of interest. Sixty patients were fed on a liberal maize dietary for twelve months, during that time four certain and one doubtful case occurred; sixty other patients received a diet containing no maize, five certain cases and five doubtful developed among them.

Note. A work (*Pellagra*, by H. V. Harris, M.D., Atalanta, U.S., Macmillan, New York, August, 1919) has come into the writer's hands since the completion of this paper. The author gives a valuable historical summary of existing knowledge in all branches of the subject up to the year 1915. In regard to etiology, the maize theory in a slightly modified form is strongly supported. It is suggested that the disease is due to: (a) an intoxication with "certain phenol poisons" produced by the action of moulds on the cereal; (b) that the intoxication is inherited and that possibly all pellagrins have this hereditary taint. The hypothesis is given in full on p. 120 of the book. Eleven reasons are advanced (p. 118) in support of the assumption, but no facts are adduced which are not open to considerable doubt or do not admit of a different interpretation. Zein is described as a tox-albumin. Numerous reported cases, where maize was known not to have been consumed, are explained as being either not true pellagra but a condition resembling it described by the author as "para pellagra"; or on the supposition that the patients had an ancestral taint. It is also suggested that rarely the moulds producing the toxin may grow on other cereals.

F. M. Sandwith (1913), who had for many years supported this theory of pellagra, suggested that the deficiency in maize might be explained by the lack of tryptophane in its chief protein zein,

The disease is traced by Casimir Funk (1913, 1914) to a deficiency similar to that to which he attributes beri-beri, an insufficiency of vitamins in the food.

Voegtlin (1914) draws attention to the importance of investigating: (a) the possible connection with a deficiency of certain accessory food substances (vitamins); (b) the toxic effects of aluminium salts; (c) the deficiency of appropriate amino-acids.

The essential curative character of high value protein in the treatment of pellagra is illustrated by the experience of Lorenz (1914) in advanced

mental cases, Ridlon (1916) and in particular by the orphanage experiments of Goldberger, Waring and Willets (1915); (see also Goldberger (1916)). The results obtained were so striking that they may with advantage be quoted. In two orphanages, M.J. and B.J., there had been frequent recurrences of pellagra: the number of cases between January and September 1914 was at M.J. 79, at B.J. 130. The diet appears to have consisted largely of cereals, partly maize, with very little animal food; from September 1914 onwards oatmeal replaced grits, a considerable increase in the meat was made, leguminous food which had before been absent was introduced and milk and eggs were given.

Not only, in fact, was the diet improved in quantity but it is clear that a large amount of protein of high biological value was added. The result in 1915 was that at M.J. no case occurred, at B.J. one recurrence.

In similar orphanages at which the old diet was continued recurrences occurred to the extent of from 58 to 75 %.

A like result was seen at the Georgia State Asylum. A ward was set apart for pellagrins, the patients in this ward received an extra half-pound of meat in addition to milk and leguminous food. No recurrences took place, while in the control wards, in which the improved diet was not given, recurrences were among the white patients 53 %, among the coloured 40 %.

In neither of these cases are the components of the diets given in sufficient detail to allow of an estimate being made of the quantitative composition in proximate principles.

Goldberger (1914) advises that in pellagrous families, who cannot afford to buy sufficient animal food, leguminous food products, in view of the richness in protein, should be used.

A direct attempt to discover whether pellagra could be produced in healthy men by diminishing the protein intake was made by Goldberger and Wheeler (1915, 1916) in the Rankin Farm experiment. These authors have recently (1920) published a complete account of the composition of the diet and of the clinical symptoms which resulted. From this it appears that from April to the end of October 1915 eleven volunteers in this convict establishment were fed on a diet the average gross protein content of which was no more than 44 grammes. The writer has given a description of this diet (Diet 10, Table IX) in this paper, it will be seen that the biological value of the protein estimated on the basis of K. Thomas' (1909) investigations was 14.5, the minimum normal requirement being 30.

Control squads receiving a diet the gross protein content of which varied from 88 to 110 including 18 to 35 grammes of animal protein but being under otherwise similar conditions were observed.

The fats were large in amount, averaging 108 g. daily. After four months ~~six~~ if not seven of the eleven volunteers had developed cutaneous and general symptoms characteristic of pellagra; none appeared in any other occupant of the locality. McNeal (1916) casts doubts on the correctness of the diagnosis;

no one however with some experience of the disease can fail to be convinced, after reading Goldberger's recent paper (1920), that these men suffered from pellagra; the writer from recent experience in Egypt would be inclined to believe, from the peculiar lingual and intestinal symptoms described, that four others might well have been included as pellagrous, leaving one only out of the eleven who does not appear to have become affected.

McCollum (1919) believes, in view of the experiments of himself, Simmonds and Parsons (1917 and 1918) on animals, that the Rankin Farm dietary was probably deficient in three factors, namely the amino-acids, the inorganic salts and the fat-soluble accessory substance. Goldberger (1920) admits that in view of Sherman's (1918) observations, the low calcium content of .2 g. daily may have had an influence, Sherman considering that .45 g. is the human minimum requirement. The complete absence of xerophthalmia, shewn by McCollum to be connected with a lack of the fat-soluble accessory substance in the food, or of any symptoms of beri-beri or scurvy among the subjects of the experiment make it improbable that an absence of vitamins was the effective factor.

Experiments by Sullivan (1920) using hens fed on a diet of the same composition as the Rankin Farm dietary indicated a deficiency of the anti-neuritic vitamin B. The same observer (1920) with rats failed to get this result.

An observation which has a considerable bearing upon the points just discussed may be mentioned here.

Owing to the frequency of pellagra recurrences among the inmates of Abassia Asylum for the Insane at Cairo and the occurrence of occasional new intra-mural cases, the writer (1919) was asked to report as to whether the diet of the institution, which appeared to be adequate for normal persons, was in any way defective. The protein of the diet (No. 17, Table XVIII, this paper) will be seen to have a biological value of 46.4 (possibly 49).

The rations, in addition to other articles, contained 100 g. of meat, 50 g. of milk, and 300 g. of fresh vegetables. The calorie value was sufficient. It was thought however that as the minimum protein requirement of a pellagrous and insane community was likely to be higher, possibly considerably higher, than the normal (which the writer regards as equivalent to 40 grammes of animal protein) it would be well to increase the protein. This was done by the addition of 45 g. of meat and 50 of milk raising the biological value to about 60. During the following year the death rate from pellagra was diminished by nearly 50 %.

There was no other change dietetic or administrative. It is impossible to avoid the conclusion that the protein of the diet was raised from below the average minimum requirement of the community to something above it.

Note. The number of deaths ascribed to pellagra among the inmates of the Abbassia and Khanka Asylums, was, in 1918, 127; in 1919, 64. The death rate from all causes was in 1918, 13.4 % of the resident population. 1919, 8.2 %. The death rate from general paralysis remained unchanged.

This experience differs from others of a similar kind, such as the orphanage and other experiments described above, in that there was no change from a diet very deficient in high value protein to one very rich in such material, where it might be said that the large additions of animal food-stuffs had introduced other accessory substances previously lacking; in this case in view of the original composition of the diet it is difficult to suppose that it was lacking in either vitamins, using the term generally, or in salts of lime, or that the additions made could have added anything of great importance in these respects: there remains only the increase in the protein as the effective factor.

Chittenden and Underhill (1917) fed dogs for eight months on food consisting of biscuits, peas and cotton-seed oil: symptoms which they considered resembled those of pellagra were produced. They regard the result as due to a dietetic deficiency of unknown nature.

McCollum (1919) did not obtain any such symptoms in rats and regards Chittenden's results as due to an infection supervening on lowered vitality resulting from a faulty diet.

H. Chick and E. M. Hume (1920) carried out a series of experiments on monkeys in which all the accessory food factors (fat-soluble A, anti-neuritic and anti-scorbutic factor) were added in more than sufficient amount to the food, which was so constituted as to be deficient in the two amino-acids tryptophane and lysin, the protein consisting to the extent of 70 % of zein. Definite cutaneous symptoms were produced after periods varying from 58 to 117 days.

The addition of tryptophane to the food gave a definite beneficial effect but did not bring about a cure, the addition of a mixture of lysin, arginin and histidin gave little additional benefit in one case in which it was tried. A supplement of 5 to 10 grammes of caseinogen given with the food in the case showing the worst cutaneous lesions was followed by a rapid cure. The authors point out that the rapidity of the cure excludes the possibility of an infective agent having played a part in the condition. This important experiment seems to connect the pellagrous condition with a deficiency in the biological value of the protein and no other.

Enright (1920) in the belief that the diet of the German prisoners of war in Egypt was of high protein and calorie value finds that the epidemic (65 cases out of 7000 prisoners) among these men disproves the view that pellagra is a deficiency disease.

Goldberger (1920) and Lelean (1920) in replies to Enright's article, show that the food actually consumed by the prisoners was of very low calorie value and that the protein content was little if at all above the minimum requirement. The writer (1920) can endorse, from personal investigation of this epidemic, the opinions expressed by the two latter observers. It may be pointed out in addition that none of the 65 patients could be considered normal, all of them having suffered from malaria, dysentery or chronic diarrhoea, 62 of the 65 being affected by the two latter maladies. It may be taken

as certain that the combined effect of chronic disease of the intestine and the increased call on the protein of the food made by the insufficient energy intake, raised the level of protein requirement in these men to a point considerably above the normal.

The outbreak of pellagra among the Armenian refugees at Port Said in 1916 is of especial interest. White (1919) who has reported on this epidemic, examined with great care the epidemiology; he finds that there was no evidence of any infective origin either by insect carriers or other means.

In regard to case to case infection the epidemic is instructive in view of the statements of Siler, Garrison and McNeal (1914, 1917).

Pellagra is very rare at Port Said, the camp was a few miles from the town and was located in the desert on the Syrian side of the Suez Canal. The tent distribution of cases was carefully recorded and the results gave no evidence of the disease being either infective or contagious (*l.c.* p. 18).

[A similar conclusion was arrived at from the investigation of the outbreak among Turkish prisoners of war referred to below. A careful survey of the tent distribution of cases at the Kantara camp was made and the results submitted to Mr T. L. Bennet (Director of the Statistical Dept., Egypt); mathematically no probability was found that the occurrence of any one case influenced the appearance of any other (*l.c. infra*, p. 13).]

The writer (1916) examining the diet (Diet 7, Table III, this paper) in force at the time of the outbreak by the method first adopted in regard to the Egyptian Prison Dietaries (1914), namely on the basis of K. Thomas' observations, the validity of which method will be discussed below, was led to conclude that the low biological value of the protein showed the most obvious deficiency. This for the whole camp was equal to about 21 and for adults to 23 grammes of caseinogen daily. The rations contained a preparation of the whole wheat grain and a fair proportion of fresh vegetables, this was thought to exclude a vitamin deficiency. The view adopted as to the protein deficiency was confirmed by the beneficial results of improvements in the food, the biological value of the protein content being raised from 23, first to 37.7 (Diet 7a) later to 41.7 (Diet 8, Table VII) and for previously pellagrous persons to 59 (Diet 9, Table VIII), pellagra almost completely disappearing from the camp where, in the previous year, 20 % of the inmates had been affected.

The light thrown by the investigation of this epidemic led the writer to examine a series of other diets known to be pellagrous or the reverse; the results obtained, which form the basis of this paper, were given to the Commission which considered the outbreak of pellagra among the Turkish prisoners of war (1918) and have been published in brief in their Report by Boyd and Lelean (1918, 1920).

SUMMARY OF THE WRITER'S CONCLUSIONS.

The results the writer has arrived at, evidence of which will be given in this paper, may be summarised as follows: (a) The chief etiological factor is a deficiency of protein in the food, this is best determined by an estimation

of the biological value from K. Thomas' figures. (b) The minimum safe value of this factor is 40 below which pellagra is likely to appear in the affected community. (c) Large individual variations occur in the minimum requirement but the lower the biological value of the food protein the larger is the proportion of persons attacked. (d) The level of protein requirement is raised by labour if the energy intake is deficient, by a previous attack of pellagra and by illness especially chronic disease of the alimentary tract.

Thus while many healthy individuals may escape whose food protein has a biological value of no more than 20, the minimum safe value for a community would be 40, for hard labour probably 50 and for a community of persons with chronic intestinal disease or previously affected with pellagra as much as 60. Applying these conclusions it is thought that an explanation may be found for many of the obscure cases reported in the literature of pellagra.

Note. In a recent publication, received since the completion of this paper, Goldberger, Wheeler and Sydenstricker (1920) give an account of a valuable epidemiological survey of the relation of diet to pellagra incidence. These authors conclude that a restriction of animal proteins, meat and milk products, in the food is of definite etiological significance. They believe also that their data indicate the possibility of a vitamin deficiency, particularly in the fat-soluble A substance, being in part responsible for the disease.

In the course of an investigation carried out in Egypt during the present year (printed as a Report to the D.M.S. Egypt) Hammond Searle and Stevenson state that advanced cases of pellagra can be restored, at least temporarily, to health if placed on a dietary the biological value of the protein of which has been greatly augmented by the addition of milk proteins in a concentrated form = "Plasmon" or freshly prepared curds from which the whey has been expressed).

INTRODUCTION.

From a consideration of the literature of the subject, in part referred to above, the following statements will receive general acceptance:

- (a) That pellagra is a disease of poverty.
- (b) That the disease is found chiefly among communities whose food is mainly vegetarian.
- (c) That it affects largely communities whose staple article of cereal food is maize.
- (d) That unless the disease has reached a certain point it can be cured and recurrence prevented by a suitable dietary.
- (e) That pellagra can undoubtedly occur in persons whose food contains no maize products.
- (f) That it is almost unknown in some countries, *e.g.* Mexico where much maize is eaten. And is apparently very rare among rice-eating communities such as the Japanese and the inhabitants of a large part of India, as also in northern European regions where animal products form a staple article of diet.
- (g) That the disease appears to have almost disappeared from those parts of the south of France where it was formerly common and

has greatly diminished in northern Italy, in both cases the event accompanying a general increase of prosperity. While it is increasing in the southern states of America and apparently also in Egypt, in the former case at any rate this increase has accompanied a greatly increased cost of food without a corresponding increase of purchasing power.

- (h) That pellagra has been known to occur among children or adults whose power of obtaining sufficient and suitable food is not open to doubt. It is, however, a medical curiosity.

These seem to be the most salient general facts. Nevertheless in the literature which has been available I have nowhere been able to find any record of the composition in proximate principles of dietaries thought to have been responsible for pellagra or which appeared to have a curative effect.

(Since this was written Goldberger and Wheeler's paper (1920) on the Rankin Farm dietary has appeared.)

It is therefore of interest to put on record the constitution of a number of diets known to have been connected with pellagra and to compare this with that of others of known value in curing or preventing the disease.

The writer's object is not to discuss the various hypotheses which have been proposed regarding the etiology of the disease, but to examine the question as to whether the theory of protein deficiency does not provide a sufficient explanation.

In order to do this it is necessary to find some measure by which the protein from different sources may be reduced to a common level. The biological value of protein will be taken as this measure.

Rubner (1912, 1913) was the first to introduce K. Thomas' conception of the differing biological value of proteins into the current literature of dietetics; but, unless this observer's original description (1909) of his experiments be consulted, the impression is gained that the numerical expression of the relative value of proteins is more to be relied upon than is actually justified.

Karl Thomas' work was based on the dictum of v. Leyden (1897) which, put briefly, is that the minimum requirement of protein varies with the variety of protein. The whole of Thomas' numerous observations were carried out on himself and in each experimental period protein from only one source was present in the food, both these facts add much to the value of the conclusions. The method used is however open to question and the results, particularly in regard to maize, must be accepted with considerable doubt.

The validity of Thomas' results is disputed by some recent authors. Sherman, Wheeler and Yates (1918) state as a result of experiments carried out on a man and two female subjects that the proteins of oats, wheat and maize have the same value in human nutrition as the average protein of the usual mixed diet, and need only to be supplemented with a small amount of milk protein to be efficient in maintaining nitrogen equilibrium. The amount

of milk protein added by these observers was however as much as from 10 to 20 % of the total protein. Sherman (1920) gives details of an experiment in which a woman weighing 55 kilos maintained nitrogen equilibrium on a diet 88 % of the protein of which was from maize, the remainder from milk, the total protein corresponding to 37 g. for a weight of 70 kilos. It is to be noted that the minimum requirement of animal protein upon which these subjects could maintain nitrogen equilibrium was not known; for purposes of comparison as between different proteins this is a point of considerable importance in view of the great individual variations in this respect; the results tabulated by Sherman (*l.c.*) of 109 determinations made by different observers showing that on a mixed diet the minimum protein requirement varies from as little as 21 g. to as much as 65 g. daily with an average of 44 g.

The introduction of milk protein in addition to the single protein vitiates to some extent Sherman's conclusion as to the value of the latter. Osborne and Mendel (1918) find that if cereal proteins be supplemented by the proteins of meat, milk and eggs, less total protein is required for maintenance and growth than with the cereal protein alone. These authors (1920) also find that the rate of growth in rats for each gramme of cereal protein in the food is only half that for each gramme of milk protein.

McCollum, Simmonds and Parsons (1917) state that the value of the protein mixtures present in all the more important seeds is without exception much lower than that of milk protein. Their values differ considerably from those given by K. Thomas, protein from wheat, maize and rice they find to have 50 %, from beans 25 %, of the milk protein value. The same authors (1918) show that maize, rye and barley proteins produce growth in rats at only half the normal rate; the low value of maize protein is particularly apparent from Chart VI (Lot 722) in the paper referred to.

It seems clear from the results of these different observers, that, while Sherman's experiments do not in fact demonstrate the true relative value of different proteins, the results of Osborne and Mendel, McCollum and his co-workers prove that vegetable proteins have a much lower value than those from animal sources. It will be found actually that if the various diets considered in this paper be dealt with on the basis of McCollum's figures (1917, *loc. cit. supra*), the comparative value of the protein in these diets would not in most cases differ much from the estimates which have been formed on the basis of K. Thomas' values.

Under the circumstances and particularly in view of the fact that, whatever may be the true explanation, the biological value of protein, estimated as it has been, forms a reasonably accurate criterion of the pellagrous or non-pellagrous character of a diet, the writer has adhered to the method which had been adopted before the appearance of the papers discussed above.

The "biological value" of proteins from different sources was found by K. Thomas (*l.c.* p. 266) to be as follows, for the more common food-stuffs:

Beef 104, milk 100, rice 88, potato 79, pulse 55, wheat 39, maize 29.

For approximately estimating the comparative value of a given amount of protein from different sources the total quantity of each protein may be divided by the following factors: animal protein 1, rice protein 1.12, potato 1.27, pulse 1.82, wheat-flour, 2.55, maize 3.4.

The minimum protein intake which is therefore necessary for a man of 70 kilos weight under the most favourable conditions would be from animal sources 30 grammes daily, rice 34, potato 38, pulse 54, wheat-flour products 76, maize 102.

It is clear that, accepting these figures as a basis of calculation, a diet in which the gross protein content might appear to be sufficient, as for example one in which maize-flour bread formed the main component (as no doubt often happens among the poorer classes of countries in which maize is the chief cereal) might be physiologically deficient.

It must also be noted that the minimum values given are only possible if the diet in other respects than protein has a high energy value, and that the lower the total energy value the higher must be the protein intake. It will thus be seen that a subsistence diet in which a certain amount of animal protein is given may be sufficient to maintain health with a low calorie value, while a subsistence diet of the same calorie value, derived entirely or very largely from vegetable sources, might be quite unable to do so. That nitrogen equilibrium may be maintained upon an amount of protein corresponding to 30 g. of protein from animal sources is probably only possible under the favourable conditions such as it is possible to establish in an experiment and that therefore under the ordinary conditions of life protein deficiency might easily arise unless a considerable margin above the absolute minimum were allowed.

It seems also to be the case that the minimum intake of protein, as determined by experiment, is that which is sufficient under normal conditions of health and that any deviation from the normal, such as diminished absorption from gastro-intestinal disturbance even of short duration, conditions constantly occurring in ordinary life, will upset the equilibrium which can only be re-established by a considerable excess of the intake over the minimum. A safe margin is necessary for this reason.

As stated above, the figures given are for the man of a certain weight, for individuals of lower weights the requirements would be less, probably in proportion to the surface and height of the body.

Using the above facts regarding the biological value of protein in gauging the sufficiency of the protein ration in the largely vegetarian Egyptian prison dietaries, the writer (1914-1917) has adopted as a minimum, except for short periods of detention, a biological value of 40 for "light labour" and of 45 for the rations of "hard labour" convicts.

There can be little doubt that the cause of the discrepancy in the biological value of proteins from different sources is the well-known difference in the proportion of the various amino-acids which compose the protein molecule,

some of which appear to be more essential than others in the maintenance of health and growth.

The feeding experiments of Hopkins and Willcock (1907) using zein with the addition of tryptophane, and of Kauffman, Aberhalden and Bloch and others (*vide* Cathcart, *l.c.*); also the work of Hopkins and Ackroyd (1916) with arginin and histidin, are sufficient proof that, at any rate in the human being and the carnivore, nitrogenous equilibrium and eventually life cannot be maintained on a diet devoid of the more complex amino-acids, and incidentally that these cannot be synthesised in the organism and must therefore be obtained in sufficient quantity from the food and ultimately from vegetable sources.

It is clear that the more nearly a protein approaches in constitution the body protein, the less of it should be required to provide the necessary building material; it is this fact which explains the higher biological value of animal protein as human food. An examination of tables showing the constitution of proteins (see Aders-Plimmer, pp. 20-25) provides an explanation in some cases, particularly in that of zein, for the low value of the protein. In the case of zein not only are tryptophane and lysin completely lacking but two other important amino-acids, arginin and histidin, are present in unusually small proportion.

It may be possible in the future to evaluate the protein for dietetic purposes by chemical analysis, hitherto, even assuming that the whole of the amino-acids composing the protein molecule were known, their percentage proportion in different proteins has been only partially determined, a large proportion of the total nitrogen being unaccounted for in all analyses. Under these circumstances Thomas' results form an extremely useful basis for the determination of the protein food value of any given diet.

Apart from the fact that protein deficiency may, in diets of low protein content, be due to the food material yielding protein of low value, there is little doubt that in a mixed community there exist many individuals who require a larger protein intake for the maintenance of the nitrogen equilibrium than do others. There are in fact various and in some cases very large differences in the individual requirements in protein. In considering the incidence of a disease probably due to protein deficiency these individual differences are of considerable importance among a community whose food contains little more than the minimum amount of nitrogen required by the average individual.

It is of interest in this connection to examine the results of the experiment carried out by Chittenden (1905) and his four co-workers upon themselves, the purpose of which was to determine the minimum protein intake required to maintain nitrogen equilibrium with a mixed diet in some cases mainly vegetarian in nature.

Accurate details are given of the composition of the diets, the amount of nitrogen intake from each of the food materials taken daily, the total nitrogen intake and output and the nitrogen balance in each of the five observers. These

were Chittenden himself, Mendel, Underhill, Beers and Dean. The food was analysed for a period of days, varying from four to seven in different cases, in which the protein intake was insufficient to maintain nitrogen equilibrium, there being a negative nitrogen balance; and during a second period of a similar number of days during which the protein intake was slightly greater, when a positive nitrogen balance was attained. In Dean's case the second experimental period was omitted.

In order to find the comparative value of the protein intake in the different cases, an attempt has been made to estimate the biological value. The following table gives the results in the five subjects.

Table I.

Estimate of individual protein requirement from Chittenden's figures.

("Physiological Economy" in *Nutrition*, 1905, pp. 34-108.)

Subject	I	II	III	IV	V				
Body weight in kilos	57	70	61.5	65	64				
			March	June	Febr.	May	Febr.	June	Febr.		
Dates of experiments	20-25	23-27	9-14	18-24	9-14	6-12	10-14	1-4	9-14
Absorbed protein in grammes	40	36.6	48.9	51.2	55.5	68.7	55.2	42	55.2
Biological value of protein	24.4	22.6	22.4	29.7	29	38.1	34	28	25.2
B.V. \times 70	29.9	27.7	22.4	29.7	33	43.3	36.7	30.1	27.5
body weight
Nitrogen balance	+ .165	- .07	- 1.19	+ .38	- .953	+ .346	+ .158	- .442	- 1.555
Calorie value of food	1613	1549	1975	2440	2168	2152	2068	1785	2529
Raised to value of 70 kil. man	1980	1901	1975	2440	2470	2450	2216	1921	2764

The nature of the diet in the five cases was as follows:

- I. Chittenden. Diet mixed, with meat and other animal protein.
- II. Mendel. Mainly vegetarian. With milk, cheese and eggs.
- III. Beers. Mainly vegetarian. With a good deal of milk and milk products.
- IV. Underhill. Mixed diet with meat and fish daily.
- V. Dean. Vegetarian with no milk and little milk products.

The method adopted in making the calculations from Chittenden's data was the following. Taking the nitrogen values given for the components of the daily diets, the nitrogen from all animal sources, rice and potatoes, is summed and multiplied by the factor 6.25, giving a certain figure; the nitrogen from all other sources (excluding tea and coffee) is similarly converted to protein, giving a second figure which, added to the first, would represent the gross protein of the diet (not shown in the Table). To obtain the absorbed (nett) protein (line 4, Table I) 5 % is deducted from the first figure, 20 % from the second and the results added. The biological value (line 5, Table I) is estimated from the absorbed protein; the first figure (protein from animal sources, rice and potato) being given the unit value of meat protein, the second figure (protein from other sources) being reduced to the value of wheat protein by dividing by the factor 2.5, the two results being added together.

It is obvious that the figures cannot be more than perhaps a close approximation, for example some of the articles of diet contain mixtures of animal and vegetable protein, for example chocolate pudding; in such cases an estimate has been made on the basis of the known composition of such mixtures and the protein entered under the two heads. While in some cases the value of the protein on the basis of Thomas' figures is unknown.

The figures from all the five cases having been treated in an identical manner the figures are comparable and demonstrate clearly enough that after reducing the whole protein to the value of meat protein the quantity necessary to maintain nitrogen equilibrium differs very considerably in different individuals living under otherwise similar conditions.

To obtain a more exact expression for comparison of the five cases the figures tabulated above have been treated thus.

To the biological value of the protein in the five cases in which there was a negative nitrogen balance has been added the value of the body protein metabolised in each case respectively as estimated from the excess of the nitrogen output over the intake, the figures so obtained are assumed to give the total amount of protein metabolised. The following table gives the results:

Table II.

Subject	I	II	IV	V	III
Body weight in kilos	57	70	65	64	61.5
B.V. of assimilated protein A (grammes)	22.6	22.4	28	25.2	29
Nitrogen balance	-.07	-1.19	-.442	-1.355	-.952
Body protein metabolised B...437	7.4	2.7	9.4	5.95
A + B total as animal protein	23.3	29.8	30.7	34.6	34.9
Ditto per kilo body weight4	.42	.47	.52	.57

The last line gives the grammes of animal protein metabolised per kilo of body-weight and shows a minimum of protein consumption daily .4 g. per kilo in the case of Chittenden and of .57 g. in the case of Beers which would correspond for a man of 70 kilos to 28 grammes in the first case and 39.9 grammes of protein in the second, this being the minimum requirement in the two cases.

Accepting the above figures as approximately expressing the true value of these diets in animal protein, they show very well that taking a community of persons receiving a certain diet as for example in a camp or institution, the diet which might be sufficient in protein for the majority of the inmates might be actually deficient for some.

A hypothetical case may be suggested.

A diet containing about a 100 g. gross protein equal to 80 g. available protein. If this should consist to the extent of 40 g. maize protein, 10 g. wheat protein, 20 g. leguminous protein, 6 g. animal protein and 4 g. rice protein, it would have a biological value of 37.7 g. Such a diet while being sufficient to maintain nitrogenous equilibrium in the majority of individuals

of ordinary weight might lead to gradual protein starvation in a certain number, although the protein intake is by no means small.

In institutional diets, at any rate in countries such as Egypt where vegetable foods are bound to supply the greater part of the nutriment, the apparent value of a diet is apt to be deceptive; in such cases it is particularly necessary to adopt some method, such as has been used above, for the purpose of gauging the value of protein. Even then it is difficult to avoid approaching the boundary line beyond which a few exceptional individuals will be found who are likely to suffer; it is for this reason that the writer (1917, *l.c. supra*) suggested that in a vegetarian diet the protein should have a biological value equal to not less than 40 grammes of animal protein.

In considering such institutional diets where the rations are based upon the absolute requirements in essential components a deficiency of protein may arise from defects in the mode of preparation owing to which the absorption of protein from the alimentary tract is insufficient, the protein thus not being available for nutrition to the normal extent.

Part I.

ANALYSIS OF VARIOUS DIETS WITH THE INCIDENCE OF PELLAGRA AMONG THE AFFECTED COMMUNITIES.

With this introduction as to method, the writer proposes to examine certain diets, some of which are thought to have had a causal relationship to pellagra, others which are known to have been curative in the disease. Apparently such a comparison has not hitherto been made, nor is there in the literature at the writer's disposal any detailed analytical treatment of pellagrous or anti-pellagrous diets.

1. ARMENIAN REFUGEE EPIDEMIC.

The first diet is that of the Armenian Refugees' Camp at Port Said. These persons to the number of nearly 4000, after undergoing great hardships in the mountainous coast region between northern Syria and southern Asia Minor, were rescued and carried to Port Said where they were received in September 1915. After an organisation for their relief had been established, they were put on the diet¹ described below. In May 1916 pellagra² was found to exist among the refugees, and owing to the large amount of sickness in the camp apparently due to this cause, Dr R. G. White of the Public Health Department (Egypt) was sent to investigate the epidemic; about 380 cases of the disease were found, almost exactly 10 % of the population being affected.

¹ This diet was introduced in April but was preceded by two very similar diets of almost identical value.

² For some months previously, diarrhoea and digestive troubles had been very prevalent.

*Diet Factor in Pellagra***Diet 7¹.**

At the beginning of June the Diet Sheet was submitted to the writer for examination. The mean daily ration and nutritive value of the same was as follows:

Table III.
Armenian Refugees' Diet. Port Said, 1916.
(Diet 7)

Article of diet	Daily amount in grammes	Gross protein	Available protein	Biological value of protein	Gross fat	Available carbohydrates	Salts
Bread*	750	—	37·5	15	7·5	356	9·7
Bourghoul†	5·5	—	·5	·2	·11	3·5	—
Cheese	17·1	—	2	2	2·7	—	1·7
Meat	8·6	—	1·6	1·6	·34	—	·1
Oil	5·3	—	—	—	5·3	—	—
Lentils	11	—	2·1	1·17	·22	6	·25
Beans	7·1	—	1·3	·7	·14	3·8	·2
Rice	8·6	—	·56	·5	·03	6·5	·03
Sugar	18·8	—	—	—	—	18·8	—
Vegetables	53·4	—	·53	·25	—	1·6	·5
Onions	2·5	—	·03	·01	·05	·07	·8
Olives	14·3	—	·11	·05	2·8	·7	·7
Value of diet for adults }		57	46·2	21·4	19·2	397	14·1
(over 14 years) }		64	51·5	23	21·6	430	—

* Wheaten with 25 % maize flour. 750 g. to persons over 14 years of age. 4-14, 375 g. 0-4, 125 g.

† Crushed wheat, boiled in water and afterwards dried for storage—a sort of porridge being made from it.

The writer (1916) presented a report in reference to this diet, the conclusions arrived at being that the cause of the epidemic of pellagra was to be found in:

1. The low biological value of the protein content of the food.
2. The low total energy value.
3. The low fat value.

Conclusions 1 and 2, in so far as the calorie value has a bearing on the maintenance of nitrogen equilibrium, appear to be confirmed by the subsequent investigations recorded below. The more in fact that this epidemic is considered, the more strikingly it stands out as an example of the production of pellagra by the use of a diet of low protein value.

Since this report was written, by the courtesy of Dr White, Dr Devletian and others it has been possible to obtain some information as to facts concerning this diet.

It would seem that the rations were not originally introduced as forming a complete diet, it being expected:

(a) That a sufficiency of other food would be forthcoming as the result of help in money or in kind from charitable sources.

¹ The numbers given to diets, e.g., Diet 7, refer to their order in the "General Table of Diets," Table XX.

(b) That the refugees, at any rate the men, would be able to obtain work at a wage which would enable them to provide sufficient food for their families.

(c) That failing the above the fact that a full diet was issued for each individual in the camp irrespective of age would lead to the deficiency in the case of the adults being balanced by the excess in the case of the children.

The writer is informed that as regards (a) the general population of the camp were little benefited, pregnant women, nursing mothers and young children, as was natural and right, receiving chief attention.

As regards (b) it appears that work was irregular and that although some families benefited many did not, while in many cases the men were inclined to save what money they received rather than spend it for the support of their families.

There can in fact be no doubt that the bulk of the population lived solely on the food provided in the diet sheet given above.

The third point (c) is capable of analytical investigation.

Unfortunately the only part of the full ration issued without regard to age was the food material other than bread.

The bread was issued thus: 750 g. to persons over 14 years, 375 g. to persons over 4 to 14 years, 125 g. to children up to 4 years.

The age distribution in the camp was in June 1916 as follows:

Table IV

(Figures kindly given by Dr White)

	0-5	6-14	15-20	21-30	31-40	41 upwards
Males ...	265	470	371	199	137	315
Females ...	253	480	372	338	236	404

From these figures the following estimates have been made:

Total population	3840
Children 0-4 years	468
„ 4-14 „	1000
Males 15 years upwards	1022
Females „ „	1350

The mean food requirements at the different ages are as follows estimated from Rubner's tables and the tables of *American Household Requirements* (from Russell-Wells, 1915):

First 4 years of life	32 % of adult male requirement
5 to 14	60 % „ „ „
Over 15 (women)	80 % „ „ „
Over 15 (men)	100 % „ „ „

Taking the age distribution in the camp and distributing the available food in the above proportion according to age, the following table gives

the calorie value and protein (calculated as animal protein) which each group would have received:

Table V.

Age				Calories	Protein. B.V.	Fat	Carbohydrate
Males 15 upwards	...			2160 (gross 2280)	23 (gross 64)	21.6	430
Females 15 upwards	...			1728	18.4		
Children 5-14		1290	13.8		
„ 0-4		690	7.3		

The above table shows that for the different ages the diet, as far as the calorie value is concerned, provided a bare subsistence for the first age group and is deficient even in this respect for the others as compared with any known figures.

The protein is more strikingly deficient.

The proportionate age requirements given above are not applicable to the protein, the daily amount needed to maintain health and growth being relatively greater.

A child of eight months naturally fed receives about 16 grammes of animal protein daily; this may be taken as the physiological need at that age. The requirement gradually increases with age; it has been suggested above that 40 g. should be the minimum biological value of protein for the adult, the estimated minimum protein requirements (estimated as animal protein) for the different age groups would be thus in the order of the above table, 40 g., 32 g., 25 g., and 20 g. daily; with lower figures than these it is doubtful whether health and growth could be maintained over any considerable period of time.

Comparing these figures with those given in the table (V) showing the value of the food actually distributed, the general condition of protein starvation becomes obvious.

No doubt some food was available from other sources, to this reference has been made above. There is however every reason to suppose that the married women with families, tended in this camp as happens in ordinary life under conditions of poverty to sacrifice part of their food to the feeding of their children and husbands; persons familiar with the Armenian peasants, from whom the population of the camp was drawn, state that this is particularly likely to have been the case with the women of that race. When we correlate three factors, poverty (or a restricted food supply), the maternal instinct and the care of a household with a disease due to deficient nutrition, that disease might be expected to attack the married women to a greater extent than other classes.

The distribution of the cases occurred very strikingly in the camp and is equally marked in the case records given of the Spartanburg epidemic by Siler and Garrison (1914) and elsewhere in the United States by Grimm (1916).

A table constructed from case records obtained from Dr Devletian is of interest in this connection.

Total population of camp 1916 (June), 3790.

Total cases of pellagra 1916, 643.

Table VI.

			Number	Pellagra cases	% affected
Males 0-14	735	54	7.4
Females „	683	66	9.8
Males over 14	1022	77	7.6
Females „	1350	445	33
Males 15-50	812	34	4.2
Females „	1071	329	31

The figures for adult males between 15 and 45 are affected to some extent as the majority of these men left the camp for work elsewhere in September 1916; as however the bulk of the cases occurred before this, the effect on the estimate is not great.

The age distribution of the cases, compiled from Dr Devletian's statistics, and a similar record of the Spartenburg cases is shown in Chart I. The similarity in the two curves is striking.

Diet 7a.

In view of the great deficiency in the diet, various recommendations were made in collaboration with Dr White and in November the following diet was introduced:

Bread 770, meat 10, oil 18.7, rice 31.2, lentils 50, beans 75, fresh vegetables 100, onions 12.5, cheese 7.1, wheat 14.8, sugar 20, salt 15, tea 20.

The figures represent the mean daily amount in grammes.

The nutritive value of the diet was as follows:

Protein (av.) 60.2, B.V. 37.7, fat 35.2, carbo-hydrates 498.

Calories (av.) 2676, salts of food 13.45.

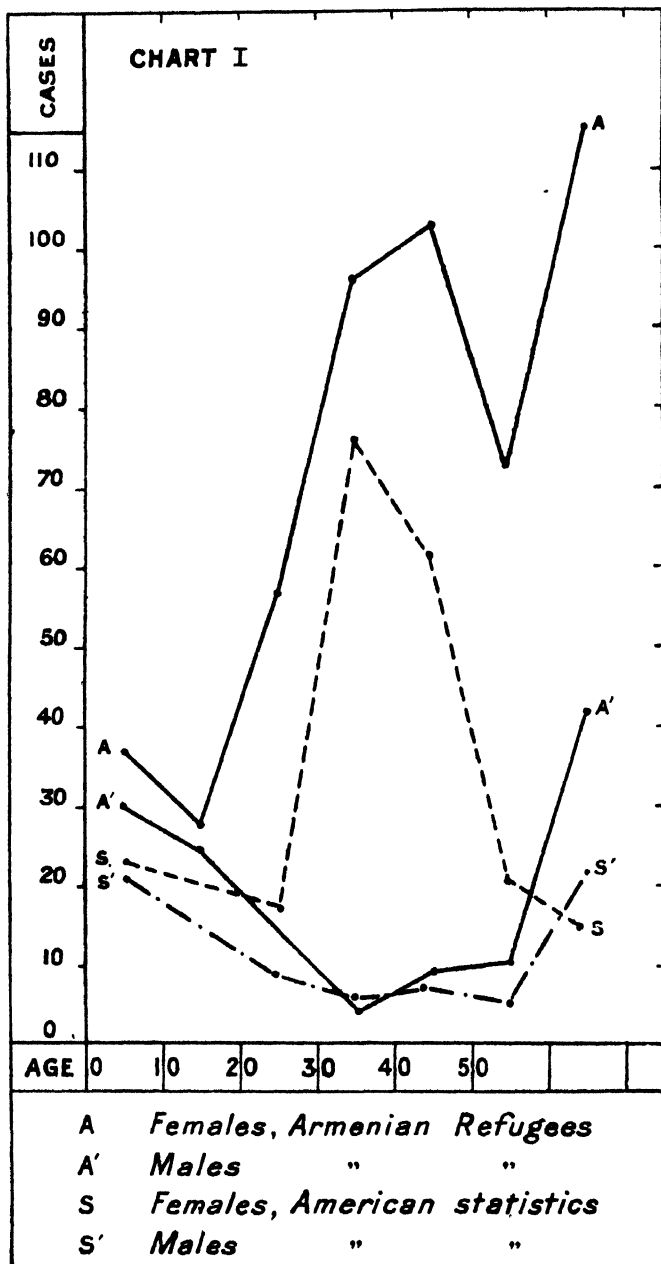
Considering that more than half of the protein value is derived from other sources than bread compared with only 25 % in the old diet, the age-distribution of the protein would have been sufficient.

This diet was in force for two months. Unfortunately, owing to national or other prejudices, the bean ration was disliked and the following diets (8 and 9) were introduced in February 1917.

The two diets were eminently successful.

Diet 8 was general for all adults in good health and not having suffered from pellagra. It is not perhaps surprising that no cases of pellagra occurred on this diet if it be considered that the persons receiving it had escaped the disease in the previous year when consuming the much lower diet then in force.

Diet 9 was introduced as an anti-pellagrous diet and was given to all persons who had suffered from pellagra in the previous year or who showed symptoms which might in any way have been connected with the disease. It is a striking testimony to the curative effect of a diet of high protein value that the last case of pellagra left hospital in June and although some rare relapses occurred in 1917 and 1918, the disease practically ceased to exist in the camp. This diet may in fact be said to be a true anti-pellagrous diet

*Diet Factor in Pellagra***AGE DISTRIBUTION ARMENIAN REFUGEES
WITH AMERICAN STATISTICS ***

* 1917 Progress Report, Thompson McFadden Commission, p.32.

1. Age and sex distribution of pellagra. Armenian refugee epidemic with comparative figures of the Spartanburg cases recorded in the Thompson McFadden Commission Report.

Diet 8.

Table VII.

Armenian Refugees' Camp. Port Said.

Diet for healthy adults. February 1917.

Articles of diet	Daily amount in grammes	Gross protein	Available (nett) protein	Biological value of protein	Gross fat	Carbo- hydrates
Bread (wheaten)...	675	45	34	13.6	6.7	310
Meat (- bone) ...	42	8.4	8	8	2.1	—
Tripe or fish ...	60	10.5	9.6	9.6	3	—
Lentils ...	50	12	9.6	5.2	1	27.2
Rice ...	50	3.7	3.7	3	.2	38
Bourghoul ...	50	6	4.7	1.9	.9	34
Vegetables ...	150	2	1.5	.8	—	4.5
Oil... ...	20	—	—	—	20	—
Suet ...	10	—	—	—	7.5	—
Onions ...	15	.2	.18	.09	.03	.42
Cheese (skim-milk) ...	12	1.8	1.5	1.5	—	—
Olives ...	7	.08	.06	.03	1.4	.35
Halwa ...	7	.1	.07	.03	1.8	4.6
Sugar ...	20	—	—	—	—	20
Salt ...	15	—	—	—	—	—
	—	90.6	72.5	43.7	46.1	439

CALORIES: gross, 2693; available, 2513.

Diet 9.

Table VIII.

Armenian Refugees' Camp. Port Said.

Anti-pellagrous diet for sick or ill-nourished.

Articles of diet	Daily amount in grammes	Gross protein	Available (nett) protein	Biological value of protein	Gross fat	Carbo- hydrates
Bread (wheaten) ...	600	40	30	12	6	287.5
Meat (- bone) ...	42	8.4	8	8	2.1	—
Tripe or fish ...	60	10.5	9.6	9.6	3	—
Eggs (1) ...	40	4.5	4.4	4.4	4	—
Lentils ...	33.3	8	6.4	3.5	.7	18.2
Rice ...	33.3	2.8	2.2	2	.1	25.3
Bourghoul ...	33.3	4	3.2	1.3	.6	22
Vegetables ...	100	1.2	1	.5	—	3
Onions ...	15	.2	.18	.09	.13	.42
Butter ...	30	—	—	—	27	—
Oil... ...	15	—	—	—	15	—
Milk (buffalo) ...	350	19	17.5	17.5	28	14
Olives ...	7	.08	.06	.03	1.4	.35
Halwa ...	7	.1	.06	.03	1.8	4.6
Sugar ...	60	—	—	—	—	60
Jam ...	14	.5	.4	.2	—	8.4
	—	99.3	83.0	59.15	89.8	443

CALORIES: gross, 3143; available, 3002.

in that it not only prevents the occurrence of primary cases but is also sufficient to prevent relapses.

The diet (9) is drawn up on lines very similar to those of the various diets which have been found to be anti-pellagrous in the numerous orphanage and other institutional experiments which have been carried out by Goldberger and his co-workers (1915) and by Ridlon (1916) in the United States. It was

CHART II
INCIDENCE OF PELLAGRA AMONG ARMENIAN REFUGEES
UNDER INFLUENCE OF ORIGINAL AND IMPROVED DIETARIES

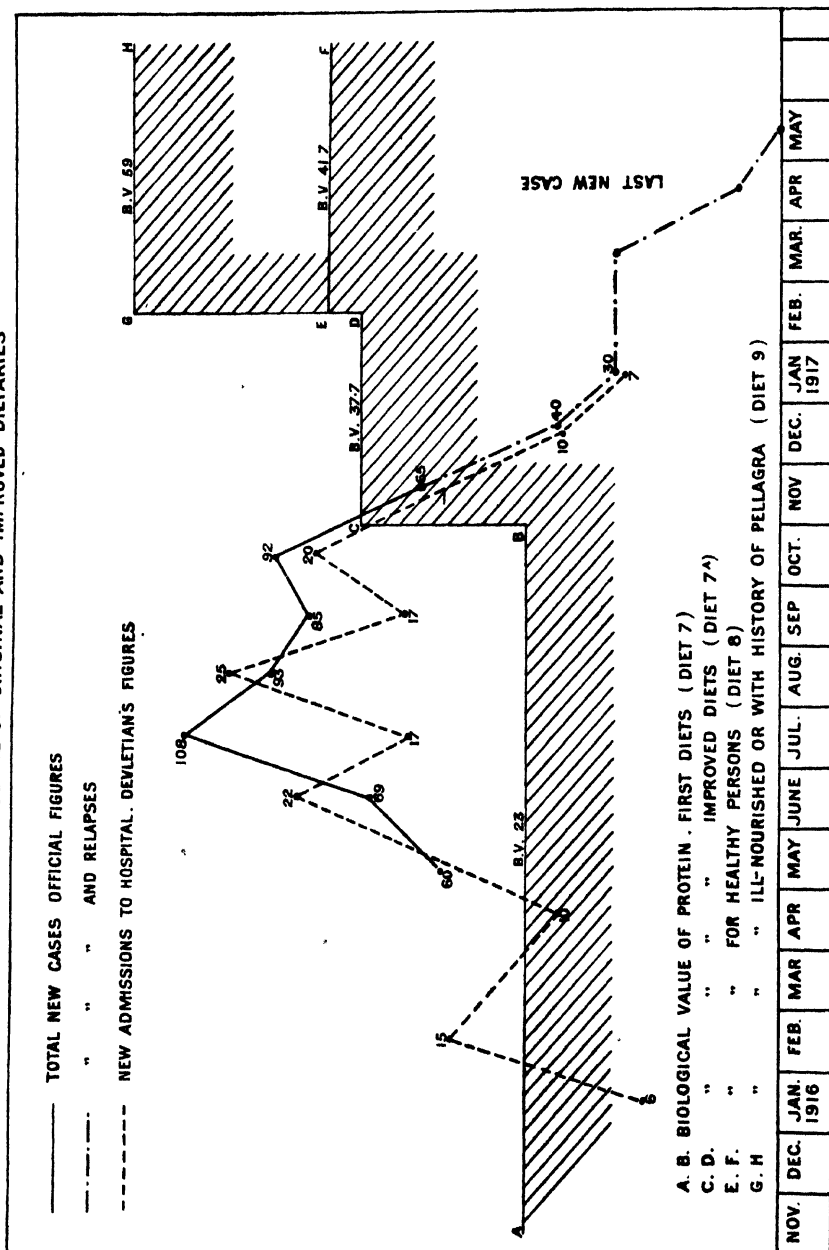


Chart 2. Relation of the biological value of the protein in diets 7, 7a, 8 and 9 to the monthly pellagra incidence in the Armenian refugee epidemic.

as successful as these latter, for although a few cases were seen during four months after its introduction, no cases of pellagra occurred after June 1917. It will no doubt always be found that a generous diet rich in readily assimilated proteins and in fats allowing therefore a large margin for the defective digestive powers of the pellagrous or persons susceptible to that disease, will prevent its appearance; it is however also probable that such cases would again relapse if placed on an ordinary diet which would be amply sufficient for normal individuals. Whether a diet as rich as this in proteins but containing only a small proportion of fats would be equally good there is no direct evidence to prove, all the anti-pellagrous diets employed being characterised not only by their richness in readily assimilated protein but also in animal fats. There is however evidence to show that diets in which the proteins are of fair biological value but the fats very small in amount are not pellagrous. Certain diets (Nos. 19, 21, Table XX) are given later as evidence of this.

The relations of the incidence of pellagra to the biological value of the protein in the above diets (7-9) is shown in Chart II constructed from the official figures of the total cases of pellagra known to have occurred in the camp from May 1916 when the disease was diagnosed until April 1917, when the last case entered hospital. For these figures the writer is indebted to Lt-Col. Lelean and Dr Devletian. In the Chart the monthly incidence is plotted against the biological value in protein of the diets consumed by the refugees during the period January 1916 to June 1917.

2. RANKIN FARM EXPERIMENT.

Diet 10.

The second diet which is known to have had a direct causative relation to pellagra is that administered in the experiment on eleven convicts by Goldberger and Wheeler (1915) at the farm of the Mississippi State Penitentiary, Jackson, Miss.

Goldberger gives the total quantities of cooked food consumed during one week (ending August 8th, 1915) by the experimental squad; from his figures an estimate has been made of the composition and the nutritive value of the mean daily diet as set out in the following table. Leguminous food and meat (except 4 oz. on one day in the week) were excluded.

It is not until the protein of this diet has been estimated on the basis of the biological value that the extreme deficiency of the protein is brought out quantitatively. The biological value is seen to be no more than 14.6 grammes daily. The body weights of the men varied from 56.4 to 86.4 kilos—average 67.7. The biological value of the protein absorbed daily appears to have averaged only .215 gramme per kilo with a minimum in one man of .17. The lowest of the five cases recorded by Chittenden (see p. 10) was .4 in a man of 70 kilos. The energy value was probably sufficient for the type of

labour; the result cannot therefore be ascribed to deficiency in that respect as might have been suggested from an examination of the Refugee diet (7).

Table IX.

Mean composition of Diet 10. Rankin Farm experiment.

Articles of diet (1)	Daily amount in grammes	Gross protein	Available protein (2)	Biological value of protein (3)	Gross fat	Carbo-hydrate
Butter-milk	10.2	.306	.306	.306	.05	.49
Corn-meal	212	19.5	15.6	4.6	4	160
Grits	35.3	3.3	2.7	.8	.25	28
Corn-starch	19.7	—	—	—	—	17.7
Wheat-flour	134.2	14.5	11.5	4.6	1.47	100
Rice	22.9	1.83	1.5	1.4	.06	18
Cane syrup	37.3	.18	.17	.8	—	28.2
Cane sugar	62.4	—	—	—	—	62.4
Sweet potatoes	97.3	1.75	1.4	1.1	.68	26.6
Turnip	6.3	.08	.06	.03	.01	.51
Turnip-greens	22.3	.94	.7	.35	.13	1.4
Cabbage	34.3	.54	.4	.2	.1	1.9
Collards	17.8	.8	.6	.3	.1	1.1
Pork fat	101.4	.09	.09	.09	101.2	—
	813.4	43.82	35.0	14.6	108.0	446.3

Gross calorie value, 3014; available calorie value (fats and carbohydrates -5 %), 2836.

(1) The mean daily amounts are estimated from Tables XVIII-XXIV given in the detailed account of the Rankin Farm Experiment, Goldberger and Wheeler (1920), the estimate of gross nutritive value from Table of Constitution (Appendix C, p. 92). The figures agree closely with those given in Goldberger and Wheeler's Table XXV (p. 29).

(2) Estimated from Rubner's (1912) figures of absorbability of similar foodstuffs.

(3) From K. Thomas' figures: corn products protein, $\frac{1}{3.4}$; wheat, $\frac{1}{2.5}$; rice, $\frac{1}{1.1}$; potato, $\frac{1}{1.26}$; animal protein, $\frac{1}{1}$; other proteins assumed, $\frac{1}{2}$.

In this paper as originally compiled the writer made an estimate of the probable value of the Rankin Farm diet from a brief description of the food given by Goldberger and Wheeler in an earlier publication (1915). This estimate in which the gross protein appeared to be 65 and biological value 25.5 has been corrected on the basis of Goldberger's recent paper (1920), the gross protein being 43.8 and the biological value being no more than 14.5. (Sullivan's estimate (1920) from analysis of the food gives a somewhat lower value for the gross protein.)

3. INCIDENCE AMONG ITALIAN PEASANTS.

Diet 11.

A. Marie (*l.c.* pp. 316-319) gives details regarding the food of Italian peasants in pellagrous districts. The only one of these diets of which the details are sufficiently precise for analysis is that on page 318, the average daily diet of a hired peasant of the province of Ferrara; it is as follows: A. being the average for eight months of the year when labour is said to be light. B. for four months when labour is more arduous.

Table X.

Average daily food (Diet 11), hired peasant, Ferrara district, Italy.

Estimated from details by A. Marie. Lavinder's Translation, pp. 317-18.

A. Eight months of year Article of food	Amount in grammes	Gross protein	Available (nett) protein	Biological value of protein	Gross fat	Carbo- hydrates
Polenta (from maize meal)	1000	24	20	5.8	4.3	260
Egg (2 each week) ...	12	1.5	1.5	1.5	1.2	—
Onion	30	.5	.4	.2	.5	1.2
Wheat-bread	50	3.6	2.8	1.12	.5	23.5
Maccaroni	50	6.7	5.4	2.16	.4	37
Meat (pork)	10	1.8	1.7	1.7	2	—
Cheese	5	1.8	1.8	1.8	.9	—
Beans	150	34	23.5	13.1	3	81
Fish	20	3	3	3	1	—
		76.9	60.1	30.4	13.8	402.7

CALORIES: gross, 2174; available (nett), 2020.

Table XI.

Average daily food, hired peasant, Ferrara district, Italy.

B. Four months of year. Article of food.	Amount in grammes	Gross protein	Available (nett) protein	Biological value of protein	Gross fat	Carbo- hydrates
Polenta	160	3.8	3.2	.9	.6	40
Egg	30	3.9	3.9	3.9	3	—
Onions	60	1	.8	.4	1	3
Wheat-bread	400	27	20	8	4	190
Maccaroni	200	26.8	21.5	8.6	1.8	148
Meat (pork)	60	10.8	10.3	10.3	12	—
Cheese	20	7.4	7.2	7.2	2.8	—
Beans	40	9	6.4	3.5	.8	22
		89.7	73.2	43	24.4	403

CALORIES: gross, 2333; available, 2164.

Note. Polenta is a form of thick gruel or porridge made by mixing maize meal with about twice its weight of boiling water the meal being scattered into the water while boiling. The mixture contains about one-third its weight of maize-meal. The beans are assumed to be the ordinary variety (*Vicia faba*) with which the writer is familiar as a food material in Egypt, having a somewhat higher protein content than many other leguminous foods.

It is also assumed that the weight given is that of the dry uncooked beans. If as is possible the weight represents that of a dish of cooked beans the nutritive value would be $\frac{1}{2}$ of that given.

It is probable that the protein of the pork is over-estimated and the fat under-estimated. The value therefore given for the protein of the diet is likely to be a maximum.

The protein value of the diet is low, the fat content exceptionally small and the energy value considerably below that for persons doing even light labour.

In diet B the protein value is probably sufficient; the energy and fat values are still low. The comparatively high protein value of diet B would not prevent the deleterious effect of the deficiency of diet A, extending as

it does continuously over eight months of the year. The low energy value in A would accentuate the ill-effect of the deficient protein.

It will be remarked that the biological value of the protein in the Ferrara peasant diet (diet 11) is considerably greater than in the case of diets 7 and 10, the incidence of pellagra was however much less. According to the census of pellagrins 1905 (Marie, *l.c.* p. 60) there were in the Compartment of Emilia of which Ferrara is a province 3357 known cases. The population of the whole Compartment was in 1901, 2,477,697 (Ashby, 1911), giving an incidence rate of about .14 % for the whole population, it may be safely assumed that the peasants in the province of Ferrara were not affected to the extent of more than 3 %, while the population subjected to diet 7 (Armenian refugees) were affected to the extent of approximately 20 % and in the case of diet 10 (American convicts) at least 54, possibly 91 %.

4. INDIVIDUAL CASES AMONG EGYPTIAN FELLAHIN.

Diets 1-6.

Three cases of pellagra have recently come under the writer's notice whose diet it was possible to verify, from villages near his house in the neighbourhood of Cairo.

(a) A boy of 11 employed on light work in the garden, receiving monthly wages, and getting his food from his home.

The family is very poor, the father making a precarious living as a fisherman. Early in March 1917, the boy complained of pain in the abdomen, he was treated with a small dose of calomel and castor oil, the appearance was that of ill-health, but no special significance was attached to the condition. On the 24th of March a distinct pellagrous rash was noticed in the usual situations, neck, forehead, wrists, hands and legs (from half-way between knee and ankles), the parts uncovered by clothing. From the 31st March onwards, the boy was given daily 240 c.c. of milk mixed with one egg (40 g.) giving a total addition to the diet of 14.5 grammes of animal protein.

It is perhaps interesting to note that there was no instinctive liking for this nutritious mixture; the boy had in fact to be watched while drinking it, otherwise he gave it to one of the dogs and on one occasion was caught pouring it away and covering up the traces with sand although the mixture was slightly sweetened by the addition of about 5 g. of sugar. The patient was told that the mixture was only milk and egg with no medicine in it but he was probably suspicious¹. He was purposely not told why he was being fed or that he had any malady, as it was feared that this might lead to his altering his food in some other direction.

No other change was made in the habits of work or food, he continued to lead exactly the same life as before.

The diet of this boy consisted of five loaves of dura (maize) bread daily

¹ Dr Dudgeon states that pellagrins generally dislike liquid nourishment and do not do well on it, this is of interest in connection with the defective gastric secretion.

with an occasional onion and some salt at midday, some raw or boiled green vegetables at supper and about twice a week some boiled beans, probably about 60 g. of beans on each occasion (60 g. being taken as the amount as this is about the quantity in an ordinary portion of "ful mademmis").

The five loaves together weighed 450 g. The loaves vary considerably in weight, they are roughly circular and about 10 inches in diameter, and of the thickness of stiff cardboard.

The bread is made from very soft unleavened dough, after baking it is crisp and biscuit-like in consistence.

A specimen of the bread was analysed, it contained 21 % of water, the dry residue containing 7.8 % of protein estimated from the total nitrogen. The bread therefore contained 6.24 % of protein; assuming that 80 % of this would be absorbed and be available for nutrition the available protein was 5 % = 22.5 g. daily, having a biological value of 6.7. Adding the protein from beans and vegetables, the total available protein was 29 g., having a biological value of 10.4 g. daily. The milk and egg raised the available protein to $29 + 14.5 = 43.5$ and the biological value to 24.9 g. daily, an amount sufficient for a boy of eleven.

The rash continued to increase slightly for two weeks, it then began rapidly to improve, disappearing first from the face and neck; traces could still be seen on the wrists as late as the 7th of May.

The boy's general condition and appearance improved coincidentally.

This case has been recorded because it seemed to be of interest to observe whether the mere raising of the protein value to a point just above the minimum by the addition of a fairly accurately known daily quantity without the variation of any other factor would be curative. In most patients who come under treatment not only is the addition to the protein much more considerable than in this case, but the patient gets rest from work and is usually removed from the direct action of sunlight.

(b) The second case is that of a boy aged 12 living in great poverty, the father having died and the mother married again. The boy lives in the house of an uncle. His food consists of five loaves daily, similar to the type mentioned above, with a certain amount of green vegetables and about 25 g. of native cheese daily. The total available protein was approximately 30 g. daily, having a biological value of 11.7 g. The rash was very marked on the exposed regions, the "Casal collar" being particularly well seen. There was some enteritis. The boy was sent to hospital where he was treated, being put on the ordinary diet (diet 18) of the hospital. He was found to be suffering from ankylostomiasis for which he was treated in the usual manner with eucalyptus oil. On May the 25th the rash had greatly improved, the general condition was obviously better.

(c) Youth aged 15 to 16 years. A very severe case, the rash being unusually intense. Considerable mental hebetude. Stated that he had not been able

to work for some time. His food consisted of six dura loaves daily with some green vegetables in the evening.

The estimated available protein 31 g. having a biological value of 10 g.

This case was admitted to Kaar-el-Aini Hospital on the same date as case (b) under the care of Dr S. Asmi. This patient was also treated for ankylostomiasis.

The urine in cases (b) and (c) contained a trace of albumin, but in neither of the three was indican present.

(Note.—There is some doubt as to the last point.)

The last two cases are probably examples of a not uncommon condition of poverty, among the poorest class of the fellahin, namely those not in constant employ on some landowner's estate but working for a precarious daily wage. The exceptionally high price of dura and the almost prohibitive price of wheat (to the poor) no doubt make the conditions particularly hard this year (1917).

An adult fellah would eat 18 or more of the loaves mentioned daily, approximate weight 1600 g. containing 80 g. available protein, having a biological value of 25 g.; in addition he would eat various vegetables, some cheese or olives, occasionally beans or lentils, possibly raising the biological value of the protein to 35 g.: it will be seen therefore that even under normal conditions a large part of the peasant population of Egypt must be living on the border-line of protein deficiency.

5. INCIDENCE AMONG TURKISH PRISONERS OF WAR IN EGYPT.

Diets 12 and 13.

These diets (Tables XII and XIII), under the influence of which a large number of cases of pellagra occurred, are of considerable interest. Diet 12 was provided for a large group (A) of men on no labour. Diet 13 for a similar group (B) with moderate labour.

H. E. Roaf and the writer investigated the metabolism on this diet in a group of five men during a period of five days with the result that it was found that the percentage loss of protein in the alimentary tract was 33 %, and of fat, 19.5 %, the available nutritive value being thus reduced to the following:

Available protein, 60. B.V., 33.5. Fat, 21.8. Calories, 2545.

This was ascribed to the coarse nature of the bread and the fact that the beans were insufficiently cooked, hard and indigestible.

With reference to a diet originally supplied to these prisoners, almost identical with this, the writer reported as follows to the D.M.S. Egyptian Expeditionary Force in April 1917:

"This diet is sufficient in calorie value for men doing very light labour. The biological value of the protein is however rather low. The minimum allowable for this factor is said to be 30, but it is wise to allow a fair margin for individual idiosyncrasies and it would probably be advisable to take 40 as the minimum.

Table XII.
Turkish Prisoners of War Diets. Egypt, 1918.
 Group A. Non-labour.

Article of diet	Daily amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo-hydrates
Bread, wheaten (10 % millet)...	785	52.5	39.2	15.6	7.8	370
Meat (with bone) ...	31.2	4.8	4.5	4.5	1.2	—
Oil... ..	15.4	—	—	—	15.4	—
Cheese (skim-milk) ...	27	5.9	5.6	5.6	.3	—
Sugar	15.4	—	—	—	—	15.4
Rice	94	7.2	6.1	5.6	.3	72
Lentils or beans ...	62.5	17	11.8	6.3	1.2	34
Fresh vegetables...	140	1.7	1.4	.7	—	4.2
Onions	15.4	.2	.14	.07	.27	.75
Dates	27	.45	.4	2	.5	15.5
Salt	15.4	—	—	—	—	—
TOTAL (normal factor of protein availability) }		89.7	69	38.6	27	511.8
CALORIES: gross, 2825; available, 2629.						
TOTAL (protein loss * 33 %, fat 19.5 %):			60	33.5	21.8	511.8
CALORIES: available, 2545.						

* From estimates of faecal N and fat, by H. E. Roaf and W. H. Wilson (1918).

Diet 13. Moderate labour. Group B.

Table XIII.
Turkish Prisoners of War diets. Egypt, 1918.
 Group B. Labour.

Article of diet	Daily amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo-hydrates
Bread (wheaten, 10 % millet)...	906	60.5	45.2	18.1	9	430
Meat + bone	114	18	17.1	17.1	4.5	—
Oil... ..	14.2	—	—	—	14.2	—
Sugar	28.4	—	—	—	—	28.4
Rice	85	6.6	5.6	5.2	.3	65
Lentils or beans ...	65	17.5	12.3	6.8	1.2	35.5
Fresh vegetables...	114	1.3	1.1	.5	—	3.4
Onions	14.2	.17	.13	.06	.25	.7
Dates	56.7	.9	.8	.4	1	31
Salt	14.2	—	—	—	—	—
TOTAL (normal factor of protein availability) }		105	82.3	48.2	30.5	594
CALORIES: gross, 3265; available, 2954.						
TOTAL (protein loss, 33 %; fat, 19.5):			70	40.9	24.5	
CALORIES: available, 2849.						
Reduced value *	—	95.5	63	36.8	22.5	535
CALORIES: gross, 2903; available, 2650.						

* For some months this diet was reduced by approximately 10 %.

"I am inclined to think that the capacity of a diet for maintaining health, particularly in regard to pellagra, is measured by this factor. If this view be correct, it would be well to raise the protein value by the addition of some extra beans or lentils. It has to be remembered that many of these men arrived in this country after a period of semi-starvation in the Hedjaz, and that to build up the body protein under such circumstances requires a diet considerably richer in protein than would suffice under ordinary circumstances; it is also to be noted that some of these men have previously suffered from pellagra, and that for such persons a diet richer in protein is probably required than would be the case for normal individuals."

The inference drawn appears to have been justified by the occurrence of a considerable outbreak among the men receiving the diet described above.

The total nitrogen elimination was examined in the urine of five men on this diet during a period of five days; the results showed that the waste of protein in the intestine was even more marked than in the first case; the absolute percentage loss could however not be determined. Assuming that this was the same as in the first case the available nutritive value of the diet was:

Available protein, 70; B.V., 40.9; fat, 24.5; cal., 2849.

During some months previous to the severest incidence of the disease, the value was still further reduced by 10 % owing to deficiencies in the supply. The nutritive value being during this time:

Protein (gr.), 95; protein (av.), 63; B.V., 36.8; fat, 22.5.

Carbohydrate, 535; calories (gr.), 2903; calories (av.), 2650.

The fact of special interest in regard to these two groups is that whereas both were rather heavily affected by pellagra there is no doubt that in the labour group the incidence was considerably greater than in group (A).

These diets are examples of ones having a protein value on the border line of sufficiency which were rendered absolutely deficient owing to the defective absorption brought about by faulty methods of preparation. (Diets 15 and 14, Tables XIV, XV.)

Note. An account of this epidemic has since been published by Boyd and Lelcan (1918, 1920).

6. INCIDENCE OF PELLAGRA AMONG CONVICTS IN EGYPT.

Diets 14-15.

The following two diets have been in use for some years in the Egyptian convict prisons for long term prisoners:

- (A) For hard labour (chiefly work in the stone quarries).
- (B) For light and moderate labour (stone-breaking, building, gardening and industrial labour).

Neither (A) nor (B) contain any dura (maize) or maize product; there can be no doubt about this as the millet from which the bread is made is milled and the flour made into bread within the prison precincts.

These two diets are of interest as illustrating in the case of (A) a diet with a protein value considerably above the minimum requirement and a calorie

value below the energy requirements, the higher incidence of pellagra in (A) being due apparently to the secondary effect on the protein metabolism of heavy work on a deficient energy supply, bearing out the conclusion arrived at as regards the group of men on diet 13.

The hard labour diet (15) has the following composition:

Table XIV.

Prison dietaries. Tura.

No. III. Diet for hard labour. (Group A.)

Article of diet	Daily amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo-hydrates	Salts
Millet bread ...	936	60	31.8	9.3	14	421	11.2
Meat (- bone)...	118.5	23.7	22.5	22.5	4.7	—	1.5
Lentils ...	56.2	35.5	24.8	13.7	2.6	71.2	4
Beans ...	75						
Rice ...	37.4	2.87	2.4	2.2	.1	28.4	.1
Onions ...	12.5	.2	.2	.1	.2	.6	.1
Fresh vegetables	100	1.2	1	.5	—	3	1
Oil ...	25	—	—	—	25	—	—
Salt ...	12.5	—	—	—	—	—	12.5
		123.5	82.7	48.3	46.6	524	30.5

CALORIES: gross, 3195; available, 2920.

The light labour diet (14) is as follows:

Table XV.

Prison dietaries. Tura.

No. II. Diet for light labour. (Group B.)

Article of diet	Daily amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo-hydrates	Salts
Millet bread ...	936	60	31.8	9.3	14	421	11.2
Meat (- bone)...	31.2	6.3	6	6	1.2	—	.3
Lentils ...	75	40.5	28.5	15.7	4	81.6	4.5
Beans ...	75						
Rice ...	31.2	2.4	2	1.9	.1	22.6	.1
Onions ...	12.5	.2	.2	.1	.2	.6	.1
Fresh vegetables	100	1.2	1	.5	—	3	1
Oil ...	25	—	—	—	25	—	—
Salt ...	12.5	—	—	—	—	—	12.5
		110.6	69.5	33.5	43.5	528.8	29.8

CALORIES: gross, 3062; available, 2854.

STATISTICS OF PELLAGRA AT TURA.

In regard to this question the pellagra statistics for the year 1917 of the Egyptian Government convict establishment at Tura are particularly instructive.

The number of prisoners varies annually, being usually rather over 2000. For purposes of labour, the convicts are divided into three classes: (Class I) light labour, (Class II) moderate labour, (Class III) hard labour, the energy value in kilogramme metres being in the three classes respectively approximately 60,000, 80,000 and 140,000. A description of the types of labour and the basis upon which the above estimate has been made will be found in the report of a committee appointed in 1914 to consider prison diets. (Interim Report of the Prisons Diets Committee, 1917, Appendix VI, pp. 29 to 39.) Prisoners on hard labour, receive diet 15 (Table XIV), on moderate and light labour diet 14 (Table XV).

The prisoners when first incarcerated are in Class III with hard labour, passing in course of years first to Class II, eventually to Class I. Men, however, who are not thought to be medically fit for hard labour may be placed at once or at any time on moderate or light labour. It would therefore have been expected that pellagra would have been least prevalent among the most healthy group. The reverse is the case.

The convicts properly belonging to Class III form 60 % of the inmates, 40 % belonging to Class II and I. After the transfer of unfit men from Class III to the lighter forms of labour, the hard labour group form only 35 % of the whole. The following table gives the distribution of labour and the incidence of pellagra:

Table XVI.

Class of labour	III	II	I	Total
Number of men	720	870	470	2060
Per cent. of total number ...	35	42	23	
Number of cases	44	33	15	92
Per cent. of total cases ...	48	35.5	16.5	
Per cent. of men (incidence) ...	6.4	3.8	3.2	
Percent. incidence after deducting } relapses from 1916 (estimated) }	4.4	2.8	1.5	

Of the cases among convicts in Classes II and I, 18 out of 33 had been transferred from Class III, as medically unfit, in the former, 10 out of the 15 in the latter, it results therefore that of men on moderate labour, who might be considered physically fit, only 15 contracted pellagra, while of physically fit men on light labour only 5 were affected.

It will be seen that convicts on hard labour receiving a diet having the available calorie value of 2920 (gross 3195) and a protein biological value of 48.5 were affected to the extent of 4.4 %; men on moderate labour on a diet having an available calorie value of 2854 (gross 3062) and protein biological value of 33.5, to the extent of 2.8 %; and men on light labour with the same diet as the last group, to the extent of only 1.5 %.

The figures are perhaps more surprising if it is remembered that the convicts of Class III may be regarded as selected for physical fitness, nearly two-thirds of Class II and a smaller proportion of Class I consisting of men found to be unfit for hard labour.

The incidence of pellagra among these three groups seems thus to be definitely associated with the relation between the energy requirement and the energy intake¹.

Diet 16.

This diet is given to pellagrous cases occurring among the convicts of all classes, the men *being taken off labour* and retained under supervision in a prison ward set apart for such cases; the seriously ill only being admitted to hospital.

The composition of the diet is as follows:

Table XVII.

Prison dietaries. Tura.

Hospital diet: with extra half-loaf of bread. Diet for pellagrous cases.

Article of diet	Daily amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo-hydrates	Salts
Bread (wheaten) (two loaves)	624	47.2	35.2	14	7	334.5	8.4
Mutton (bone) ...	94	16.9	16.1	16.1	5.6	—	1.8
Milk (Buffalo) ...	337	13.5	13.3	13.3	26	18	2.7
Lentils ...	62.5	17.2	12	6.7	1.2	34	1.7
Rice ...	62.5	4.8	4.1	3.7	.2	47	.2
Onions ...	7.5	.45	.31	.17	.67	1.8	.2
Fresh vegetables ...	156	1.8	1.6	.8	—	4.6	1.5
Oil ...	18.5	—	—	—	18.5	—	—
Salt ...	12.5	—	—	—	—	—	12.5
		101.8	82.7	54.7	59.3	440	29

CALORIES: gross, 2871; available, 2695.

On this diet the patients in most cases recover rapidly.

It will be noted that the biological value of the protein is relatively high, 54.7 as compared to 33.5 for the light labour and 48.5 for the hard labour diet; and that the protein is also in a more digestible form, mainly owing to the bread being of wheaten flour in the place of millet. The calorie value is 160 below that of (B) but is amply sufficient for men on no labour.

¹ I have to thank Dr Kirton, P.M.O., Prisons Department, and Ali Bey, P.M.O., Tura Prisons, for the list of cases, and Randall Bey, Governor of Tura, for the figures upon which the above estimates are based.

Diet 15 for hard labour is deficient to the extent of not less than 200 calories (*vide* Prisons Diets Report on the estimated energy requirement).

7. OCCURRENCE OF INTRA-MURAL CASES OF PELLAGRA AMONG AN
ASYLUM COMMUNITY ON A DIET OF MEDIUM VALUE
NORMALLY CURATIVE OF THE DISEASE.

Diet 17.

The following is the ordinary diet provided at the Egyptian Government Asylum for the Insane at Abassia:

Table XVIII.

Mean daily ordinary diet, Abassia Asylum.

Article of diet	Amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo- hydrates	Salts
Bread (wheaten) .	562	37.6	28	11.2 (? 8.2)	5.6	270	5.6
Meat with bone ...	150	21	20	20	5.2	—	1.3
Vegetables and salad ...	250	3	2.5	1.2	—	7.5	3
Onions	50	.6	.4	.2	.9	2.5	.5
Rice	50	3.8	3.2	3	.2	38	.2
Lentils or beans ...	100	27	19	10.5	2	54	3
Flour	17	1.4	1.1	.5	.3	11.4	.2
Wheat	21	2.5	1.9	.8	.35	13.8	.25
Milk (Buffalo) ...	50	2.1	2	2	4	2.4	.4
Sugar	29	—	—	—	—	29	—
Treacle	21	—	—	—	—	14	.6
Margarine (veg. fats) ...	25 }	—	—	—	—	—	—
Oil	20 }	—	—	—	45	—	—
Salt	18	—	—	—	—	—	18
Pepper25	—	—	—	—	—	—
		99	78.1	49.4 (? 46.4)	63.5	442.6	31.8

CALORIES: gross, 2910; available, 2720.

Dr John Warnock, C.M.G. (Director of the Lunacy Division, Egypt), informs me that of the large number of cases of pellagrous insanity which are admitted annually to the asylum, the majority of cases of recent origin rapidly recover. Until lately this diet appeared to be definitely anti-pellagrous in the sense that although annual relapses were not uncommonly seen among inmates admitted with the disease, intra-mural cases were only seen with great rarity.

During the past eight months (1916-1917) however twelve inmates have become affected in whom no previous symptoms have been noted and none of whom have been less than four years in the asylum. These cases were admitted with insanity not of the pellagrous type. Dr Warnock was good enough to allow the writer to see these patients. The cutaneous symptoms were in some cases exceptionally well marked; there was little wasting, some individuals being particularly well nourished. No other symptoms were observed.

The only known change in the dietary during the past year (1917) is that whereas formerly the bread was baked in the asylum from tested wheat flour,

about a year ago the bread was put out to contract and it is highly probable that owing to the present high price of wheat, the bread may have been made from flour adulterated with maize flour. The writer examined a specimen of the bread, two loaves were found to be 8 % below the contract weight, to contain an excess of water (42 %) and to be poor in protein, the content being 5.8 % gross = 4.4 % available (nett) protein. The bread had the appearance and smell of a sample of bread made from a mixture of wheat and maize flour in equal parts. It is probable therefore that the bread contained maize flour possibly to the extent of 50 %. A mixture in this proportion is very commonly used for making bread, especially among the labouring classes in towns. Taking the various deficiencies into account and assuming the presence of 50 % maize flour, the biological value of the protein of the whole diet would be reduced to 46.4 in place of 49.

As mentioned above, intra-mural cases of this kind have occurred occasionally in former years, when the dietary is known to have contained no dura flour or other maize product.

It is difficult at first sight on the basis of the composition of the diet to explain these cases. It is true that the biological value is not much above the border line, it seems however unlikely that the protein was insufficient to maintain N equilibrium in normal individuals provided these patients, who were all of them women, consumed the diet as laid down in the diet sheet.

The diet is obviously sufficient in calorie value for persons doing no work as at Abassia, it is fairly well balanced, both the protein value and fat are not too low and yet a seemingly primary pellagra occurs.

Unfortunately, as both Dr Warnock and Dr Dudgeon (Director of Khanka Asylum) point out, it is difficult to draw any conclusion from a knowledge of the ration scale laid down in an asylum; it is impossible, especially in the melancholic type of insanity common among the pellagrous, to be sure that the patients eat the food provided, in many cases almost certainly they do not, they may suffer from a lack of appetite or the food may be stolen by another patient.

It is not difficult to imagine that where, as happens in many of the insane, an apathetic condition or a distaste for food leads to a considerable part of the food being left unconsumed, a disease due to dietetic deficiency might arise just as it would in persons, who, through poverty, were unable to provide themselves with sufficient food.

In regard to such cases, Goldberger (1914, Reprint 203) quotes Motley (Georgia State Sanatorium) as having seen pellagra in inmates who had been for ten years in the institution, also Herrington (State Hospital for the Insane, Miss.) as recording cases, one after 15, another after 20 years' residence. Both these observers state that no case has ever been seen in an attendant, although in most cases they receive exactly the same rations as the patients, many of them sleeping and living in the same wards as the patients, the only difference being that they are able to purchase additional food if they wish to do so.

Dr Dudgeon (Director of Khanka Asylum), who has a large experience of pellagrous insanity, states that in his opinion, such cases of apparently intra-mural origin are in reality relapses, and that pellagra does not arise "*de novo*" in properly fed persons. He believes that "once a pellagrin always a pellagrin" and that a relapse may occur after years of freedom from the disease. As shown however by Goldberger and his co-workers, referred to above, the seasonal reappearance of pellagra in institutional cases is almost entirely prevented by a suitable diet.

There seems to be little doubt that in persons who have suffered for long or repeatedly from pellagra some permanent damage to the digestive functions results which renders necessary a higher level of protein intake than is the case in normal individuals; to this cause must be ascribed the apparent deficiency of the Abassia ordinary diet.

The possibility of the existence of three etiological types of pellagra will be referred to later.

8. A DIET OF MEDIUM VALUE USED SUCCESSFULLY IN THE TREATMENT OF PELLAGRA.

Diet 18.

The following may be recorded as an example of a diet of relatively low calorie and gross protein value which is known to be curative of the disease.

In the year 1898 F. M. Sandwith and the writer drew up a diet which has been in use since that date as the ordinary diet at Kasr-el-Aini Hospital, Cairo. It is as follows:

Table XIX.

Egyptian Government Hospital, Kasr-el-Aini. Ordinary diet.

Article of diet	Amount in grammes	Gross protein	Available protein	Biological value of protein	Fat	Carbo- hydrates	Salts
Bread (wheaten) ...	600	40.2	30	12	6	292.5	7.2
Meat without bone ...	105	21	20	20	5.2	—	1.2
Milk (buffalo) ...	200	8.3	8	8	15.8	9.6	1.6
Fresh vegetables ...	120	1.5	1.2	.6	—	3.6	1.2
Onion ...	30	.4	.3	.15	.6	1.2	.3
Melted butter (Senn) ...	20	—	—	—	19	—	—
Ground lentils...	50	13.5	9.7	5.3	1	27	1
Rice ...	75	5.8	4.8	4.5	.3	57	.3
Sugar ...	30	—	—	—	—	30	—
Salt ...	20	—	—	—	—	—	20
Pepper25	—	—	—	—	—	—
	—	90.7	74	50.6	48	421	33

CALORIES: gross, 2631; available, 2475.

Pellagrous patients recover satisfactorily on this diet with no other special treatment¹, and if it be remembered that mild cases do not enter hospital,

¹ Those familiar with pellagra will be aware that advanced cases are seen in which no treatment, dietetic or other, is of avail.

this fact would appear to be sufficient evidence that a diet not very greatly in excess of the physiological minimum requirements supplies the material to the deficiency of which the disease is due. The comparatively high biological value of the protein, considerably greater than that of the almost entirely vegetarian food of the bulk of the population, is noteworthy as bearing on the effect of this diet in pellagra.

9. NON-PELLAGROUS DIETS.

The following are some selected diets for comparison with the pellagrous diets discussed above. Some are of low calorie or fat value but all are above the minimum safe value (40) in protein if raised to the scale of a man of 70 kilogrammes body weight. (Diets 19 to 28, Table XX.)

Diet 19-20.

This diet is given by Greig (1912) as being the type of diet responsible for a disease of nutrition (epidemic dropsy). It is quoted as an example of low fat and calorie value:

Nutritive value (70 kilos scale): gross protein, 59; available protein, 50; B.V. 42.5; fat, 3.7; carbohydrate, 380; calories (gr.), 2371; calories (av.), 2240.

This is a common diet among the class of Bengali affected by epidemic dropsy. The average weight of the persons concerned is 55 kilos. The diet is remarkable for the extremely low fat value. It consists largely of rice, which accounts for the high value of the protein and in part for the poorness in fats; it is of interest to note that recently in Germany and elsewhere (Starling, 1918) dropsy has been recognised as resulting from a deficiency of fats in the food. Had pellagra been common among the population affected by this diet it seems improbable that it would not have been noted. Among the large number of cases of epidemic dropsy analysed by Greig (*l.c.*) cutaneous symptoms are mentioned in some cases, but the description of these in no way resembles that of the well-known symptoms of pellagra. The absence of pellagra in this case probably related to the sufficient value of the protein in spite of the deficiency of the diet in other respects.

Diet 21-22.

Diet of Japanese prisoners (from Atwater and Milner, 1911). The composition is not given, but from the values in proximate principles this diet must have consisted to the extent of more than 90 % of rice with probably some leguminous food-stuff or possibly fish and a little added fat.

Protein (gr.), 43; fat, 6; carbohydrate, 444; calories (gr.), 2056.
(The value given in the table referred to is 2110.)

Assuming the above composition and the body-weight as 60 kilos, the value of the food for a man of 70 kilos would be:

Protein (gr.), 51.5; protein (av.), 46; B.V. 43.5(?); fat, 7; carbohydrate, 510; calories (gr.), 2361; calories (av.), 2118.

This diet is recorded chiefly as an example of one very poor in fat with a protein value above the minimum safe value. Beri-beri is known to occur among persons subject to such diets, but pellagra has not been recorded.

Diet 23.

Diet 23 shows the mean composition of six Indian gaol dietaries estimated from figures given by McKay (1908). The values are as follows:

Protein (gr.), 99.3; protein (av.), 76; B.V. 50; fat, 34; carbohydrate, 684; calories (gr.), 3523; calories (av.), 3270.

The diet is for industrial labour. It is given as an example of a dietary containing no animal protein or fat. The protein value is fairly high. The calorie value above the energy requirement. Pellagra has not been known to occur in these institutions.

Diet 24-25. English sewing girl.

The composition in proximate principles is given by Hutchison (1916) as an example of a subsistence diet common among the poorer working women of East London¹. The actual value given is:

Protein (gr.), 52; fat, 33; carbohydrate, 316; calories, 1820.

A diet of almost exactly this composition would be obtained from the following quantities of ordinary cheap English foodstuffs:

Bread, 1½ lb.; cheese, ¾ oz.; sugar, 1 oz.; milk, 3½ oz.; dripping, ½ oz.; smoked herring, 2 oz.

Nutritive value:

Protein (gr.), 53.8; protein (av.), 47; B.V. 34; fat, 33; carbohydrate, 316.5; calories (gr.), 1824; calories (av.), 1731.

Raised to adult male scale:

Protein (gr.), 72; protein (av.), 62; B.V. 45; fat, 44; carbohydrate, 422; calories (gr.), 2563; calories (av.), 2440.

The diet is quoted to show that in European countries, especially in England, owing to the considerable amount of cheap animal food eaten even by the poorest classes, the wages of this class were at the date when the statistics were compiled no more than 3s. 9d. weekly, the food has a sufficiently high protein value to make the occurrence of pellagra improbable.

Diets 26 and 27.

These are Scotch prison dietaries (1895) No. IV Rate for industrial labour, and No. VII Rate for hard labour. (Dunlop, 1899.)

Diet 26 is given for comparison with diet 14 with which it has a close resemblance in purpose and calorie value, the fat value however being not much more than half that of the Egyptian prison diet but the protein value considerably higher.

¹ The original statistics from which Hutchison's information is derived are not given.

Nutritive value of 26:

Protein (gr.), 117·7; protein (av.), 98; B.V. 55; fat, 24·3; carbohydrate, 517; calories (gr.), 3029; calories (av.), 2750.

Nutritive value of 27:

Protein (gr.), 124; protein (av.), 105; B.V. 66; fat, 54·6; carbohydrate, 654; calories (gr.), 3709; calories (av.), 3510.

The latter is given for comparison with diet 15 as an example of a hard labour diet of sufficient protein and calorie value.

Diet 28.

This diet shows the mean daily value of the writer's food for one week. It is given to illustrate the average composition of the food of an Englishman not restricted in his choice of food and leading a more or less sedentary life, in order to show, for comparison with the other diets given, what the biological value of the protein is in such a case.

Nutritive value:

Protein (gr.), 103; protein (av.), 91·6; B.V. 80; fat, 104; carbohydrate 300; calories (gr.), 2681; calories (av.), 2593.

RÉSUMÉ OF DEDUCTIONS FROM TABLE XX

The figure 40 for biological value of the protein has been taken as the dividing line between diets which are primarily pellagrous and those which may be pellagrous relative to other causes than deficiency in the intake. Diets 1, 2, 3 (4), 7, 10, 11, 12, 13 and 14 belong to this group.

In diet 15 the protein value is above 40, the deficiency in this case being secondary; many of the recipients being unable to maintain N equilibrium under conditions of hard labour with a deficient energy intake. The effect of diet 13 appears to have been aggravated by the same condition.

Diet 17. The Asylum Diet. The deficiency may probably be traced to the existence of chronic digestive disturbances common in pellagrous subjects (of which probably achlorhydria, leading to defective utilisation and bacterial destruction, is the most important), the deficiency being secondary to the alimentary defect. Such persons clearly require a higher protein intake than the normal.

12 and 13 are examples of border-line diets as regards protein intake rendered absolutely deficient by the indigestible nature of the food due chiefly to defective preparation.

In 14 and 15, the gross protein is fairly high. The deficiency being secondary to (a) the defective utilisation of millet protein, (b) the low biological value of protein of this type.

6, 8, 9, 16 and 18 are curative diets. 6 being the diet on which case (a) recovered without any change in other conditions or work: the energy value of this diet is high.

8 is a non-pellagrous diet for normal men. 16 curative for groups on diets 14 and 15 with rest from labour.

Diet 9 may be regarded as certainly anti-pellagrous in the sense that it was not only sufficient to cure pellagra but was sufficient to prevent the occurrence of the disease in susceptible persons of the group on diet 7.

DESCRIPTION OF CHART III.

Chart III is drawn from the figures given in Table XX, the diets being given a reference number to their order in that table.

The diets are arranged from left to right in the order of their gradually decreasing protein value.

It is desired to show by this chart in a graphic manner: (a) that the only diet factor which correlates closely with the incidence of pellagra is the biological value of the protein; (b) that the

Table XX. General Table of Diets described in Part I.

Communities receiving pellagrous or non-pellagrous diets		Incidence of pellagra	Gross protein	Available protein	Biological value of protein	Protein from animal sources	Fat	Carbo-hydrate	Available calories	Gross calories
Case c, boy of 14	29	8.5	0	10.5	350	1570	1667
" b, boy of 13	35	30	11.7	0	12	305	1488	1584
" a, boy of 11-12	37	29	10.4	0	10	390	1813	1926
" ÷ 0.6 to adult male scale	61.6	48.3	16.7	0	17	650	3021	3210
" + milk and eggs (actual)	52	43	24.8	14.5	28	402	2091	2291
" ÷ 0.6 to adult scale	86.6	71.6	41.3	24	46.6	670	3485	3735
" Refugees' Camp at Port Said, May 1916	64	51.5	23	4	21.6	430	2160	2280
" " " adults, healthy, February 1917	...	+	90.6	72.5	43.7	19	46.1	439	2513	2693
" " " anti-pellagrous, Febr. 1917	...	+	99.3	83	59.1	39.5	89.1	64	3143	3443
" Rankin Farm, Experiment	...	+	43.8	35	14.6	4	108	101.2	2836	3014
" Italian peasant (Ferrara Province)	...	+	76.9	59.6	30.4	7	13.3	5.1	2047	2194
" Pellagrous group A non-labour (Turkish prisoners, Egypt, 1918)...	...	+	89.7	60	33.5	10	21.8	1.4	2545	2825
" " B hard, moderate, and light labour (Turkish prisoners, Egypt, 1918)	...	+	95.5	63	36.8	13.7	22.5	4	2650	2903
" Egyptian convicts light and moderate labour	...	+	110.6	69.5	33.5	6	43.5	1.5	2854	3062
" " hard labour	...	+	123.5	82.7	48.3	22.8	46.6	6	524	5920
" " curative diet + rest	...	+	101.8	82.7	54.7	29.4	59.3	31.6	440	2695
" Ordinary diet, 'Abassia Asylum	...	+	99	78	46.4	22	63.5	9.2	2720	2910
" " Kasr el 'Aini Hospital	90.7	74	50.6	29.3	48	421	2475	2631
Non-pellagrous diets										
Bengali diet (actual)	46	39	33.5	0	3.7	0	1757	1863
" " × 70/55 to adult scale	59	—	42.5	0	4.8	0	2240	2371
Japanese prisoners	43	39	37	—	6	—	2000	2110
" " × 70/60 to adult scale	51.5	46	43.5	0	7	—	2118	2361
Mean of six Indian gaol diets	99.3	76	50	0	34	0	3290	3523
English sewing girl (actual)	53.8	47	34	21.5	33	29	1731	1824
" " ÷ 0.75 to adult scale	72	62	45	—	44	—	2440	2563
Industrial labour, Scottish prisons	117.7	98	55	13.7	24.3	12.5	2750	3029
Hard labour, Scottish prisons	124	105	66	32.5	54.6	32	3510	3709
W.H.W. To show comparative values of better class English diet	103	91.6	80	45	104	—	2593	2681

7-9. R. G. White (*l.c.* 17).
 10. Goldberger and Wheeler (*l.c.* 1916, pp. 10-14).
 11. A. Marie (*l.c.* p. 318).
 12-13. Boyd and Lellean (*l.c.* 1918, App. viii. 61).
 19-29. Greig (*l.c.* 33).
 21-22. Ref. *Encycl. Brit.*, 1911 edition, article "Dietetics."
 23. Estimates from McKay's figures (*l.c.* 39).
 24-25. Estimates based on case tabulated by Hutchison (*l.c.* p. 32).
 22-27. Dunlop (*l.c.* 1899, pp. 15-17, 34, 84-86).

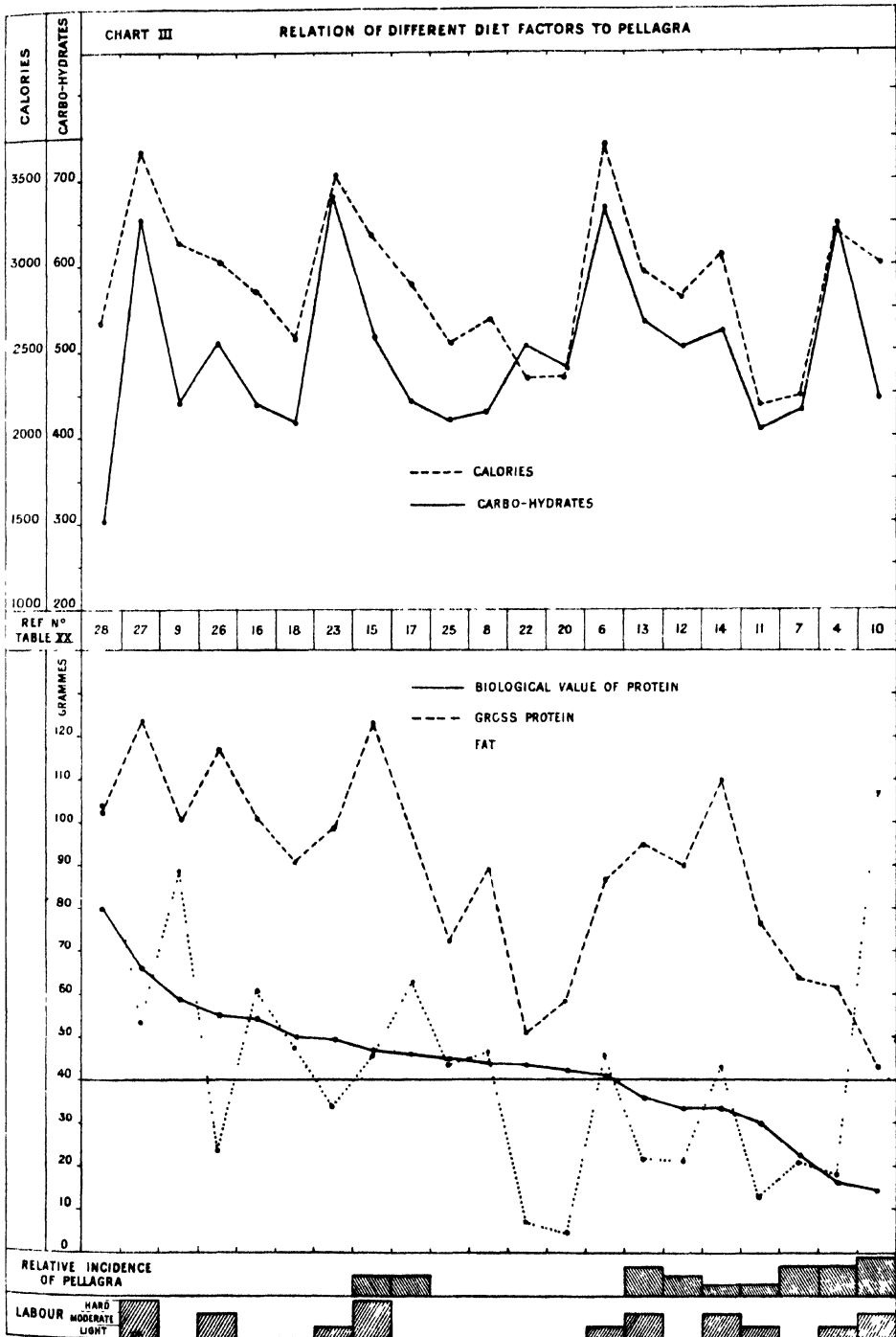


Chart 3. Relation of the different factors in the dietaries considered to the incidence of pellagra in the affected communities.

gross protein value gives little information as to the pellagrous character of a diet; (c) that labour augments the effect of deficient protein and raises the minimum protein value required to prevent pellagra.

The height of the columns showing the relative incidence of pellagra has no numerical relationship to the percentage of persons affected, but merely shows that one community was more or less severely affected than another.

Diet 4 is that of one individual, the pellagra incidence that of the class to which he belonged.

Diet 17 is that of an asylum population thought to have a high level of minimum protein requirement for reasons given in the text.

Part II.

DISCUSSION OF VARIOUS FACTORS HAVING A POSSIBLE RELATIONSHIP TO THE ETIOLOGY OF PELLAGRA.

In the series of diets recorded in Part I there are a number of factors which either singly or in combination may be responsible for the disease.

It is well to point out here that pellagra and starvation are not convertible terms; it is possible to conceive of chronic starvation on a diet containing, for example, a sufficiency of protein without the occurrence of pellagra and it is undoubtedly the case that pellagra may occur in persons who are otherwise well-nourished.

Although the tables of pellagrous diets show almost without doubt that a deficiency in the protein value is a chief etiological factor in pellagra, there are certain aspects of this question and the possible importance of deficiencies in other directions, in some cases not apparent from the known constitution of the diets, to which some consideration must be given.

These questions are as follows:

1. Deficiency in accessory food substances.
2. „ of fats and lipoids.
3. „ of protein¹.
 - (a) Absolute deficiency of intake below the normal requirements.
 - (b) Deficiency relative to individual or energy requirements.
 - (c) Deficiency due to low availability of protein resulting from the nature of the food or defective preparation.
 - (d) Secondary deficiency due to excessive bacterial destruction of protein in the intestine or defective powers of digestion or assimilation.

ACCESSORY FOOD SUBSTANCES.

The theory that pellagra is due to a deficiency in vitamins normally contained in the germ and surface-layers of maize and other cereals was first propounded by Casimir Funk (1913, 1914), the deficiency being due to the removal of these layers by modern methods of milling.

¹ The question has been already briefly discussed in the historical summary at the commencement of this paper.

This view is supported by Nightingale (1914) on the evidence of an epidemic of pellagra among convicts in South Africa who were fed on maize milled to 75 %, the epidemic disappearing when native ground millet was substituted for machine-milled maize flour. The evidence would have been more convincing if the fine maize flour had been replaced by an equal amount of hand-ground maize.

Voegtlin, Sullivan and Myers (1916) also bring forward evidence in favour of the avitamine theory (see also Voegtlin, Lake and Myers (1918)).

In none of the communities receiving the diets tabulated above was either beri-beri or scurvy (with the exception of pellagrous groups A and B, diets 12 and 13, among whom scurvy was frequent) noticed, and although, as Miss Chick (1918) has shown, the occurrence of the anti-neuritic vitamine in a particular foodstuff is no measure of its anti-scorbutic properties and *vice versa*, the fact that neither of these vitamins was deficient makes the absence of a hypothetical anti-pellagrous vitamine at least improbable.

The maize flour used in Egypt for bread-making by the fellahin is coarsely ground, little more than 7 % of the grain being removed in milling.

In diet 7 (Armenian refugees) a considerable amount of a substance, eaten largely in Asia Minor, called "Bourghoul" was used. This consists of the whole wheat grain, crushed and made into a sort of porridge, the grain being sometimes crushed, then boiled, dried and stored for use, sometimes crushed and at once made into the material as eaten. In addition these people received a fair amount of fresh vegetables including onions and tomatoes.

In the experiment on 11 American convicts (Goldberger, *l.c.*) the food contained a considerable ration of "grits" composed largely of the maize germ and in addition a quantity of fresh green vegetables.

To make the assumption probable it would be necessary to prove that there did not exist some definite deficiency in the known components of the dietary which could be correlated with the occurrence of pellagra before ascribing the disease to the deficiency of an unknown component.

FATS.

I am not aware that any author has suggested a deficiency of fats as a factor in the causation of pellagra; if however the tabulated series of diets be examined, it at once becomes apparent that the pellagrous character of these diets is, roughly, inversely proportional to the amount of fat present (with the exception of diet 10).

A deficiency of fats does not appear to have been noticed elsewhere in pro-pellagrous diets. Thus Ridlon (1916) states that in the study of dietaries consumed by his cases previous to their coming under treatment "the vegetable and fat components were notably conspicuous and the animal protein foods were relatively inconspicuous."

Lard and fat pork were used very largely by the class from which the patients came; it was owing to this peculiarity of their previous dietaries that

in the diet used for treatment of the condition, Ridlon did not allow any salt fat pork (*l.c.* p. 9).

Similarly Sydenstricker (1915, p. 29) concludes that the rise in the price of foodstuffs has restricted the supply of protein among the southern poor families to a much greater extent than it has the supply of carbohydrates and fats, and that "the proportion of proteins in the diet of the southern families is considerably less and of carbohydrate and hydrocarbons considerably more than in the diet of northern families"; it is in the former region that pellagra has become so prevalent.

The following diets from a table given by Atwater and Milner (*l.c.*) have been selected as being of very low fat value while in none of the communities or groups of men affected has pellagra been recorded, the protein value of all these diets being noticeably above the minimum value adopted as sufficient for nutrition.

Table XXI.

	Protein	Fat	Carbo- hydrates	Calories
Japanese jinricksha runner (heavy labour) ...	137	22	1010	5050
„ rice cleaner (moderate labour) ..	103	11	917	4415
Bavarian mechanic (light labour) ...	112	32	553	3060
Japanese prisoners (no work) ...	43	6	444	2110
Prussian prisoners „ ...	90	27	427	2400

The percentage of calories from fat to total calories is as follows: (1) 4 %, (2) 2.5 %, (3) 9.2 %, (4) 2.7 %, (5) 10.5 %.

In all diets which have been used for the treatment of pellagra it has been found advantageous to employ a large ration of milk and sometimes eggs (see Goldberger, Waring and Willet (1914); see also anti-pellagrous diet 9, Table VIII). These diets contain a large amount of animal fats, an unavoidable concomitant of the increase of animal protein caused by the addition of milk and eggs.

It appeared of interest in this connection to determine whether the beneficial effect of such diets was to be ascribed solely to the high biological value of the protein or whether in addition the fat itself exerted some useful influence. With this end in view, at the writer's suggestion, Col. Boyd, R.A.M.C. (1918, p. 21-22) then in charge of numerous pellagrous patients at No. 2, P.O.W. Hospital Abassia (Egypt), was good enough to carry out the following experiment.

Three groups of patients: *A*, 37 men; *B*, 10 and *C*, 10 men were fed on different diets for a period of three weeks, their weights being recorded.

A received the ordinary hospital ration containing 200 g. milk.

B received the same diet + 20 g. butter.

C received the same diet as *A* with no milk but an addition of 62 g. beans or lentils.

The caloric value of *B* and *C* was the same. The result was as follows:

Average increase in weight of				<i>A</i> = 1 lb.
„	„	„	„	<i>B</i> = 2.2 lb.
„	„	„	„	<i>C</i> = 7 lb.

It may be concluded from the above that a deficiency of fat is not directly related to the production of pellagra and that the apparent connection shown in Table XX and Chart III is accidental.

THE LIPOIDS.

The absolute quantity of fat in the diet from an estimate of the proximate principles gives no information as to the content in these essential components.

The phosphatid content of various food materials is given by Maclean (1918). The percentage of lecithin in the most important foodstuffs is as follows:

Muscle (rabbit)	0.6
Milk (cow)	0.06-0.116
Beans	0.81
Wheat	0.65

In view of these figures it is unlikely that the pellagrous character of vegetable diets has any relation to this factor.

With regard to cholesterol, J. A. Gardner and his co-workers (Gardner, Dorée, Ellis and Lander, 1907, 1909, 1912, 1914) and Lander (1915) show that this is an essential element of the food which cannot be manufactured at any rate by the mammalian organism and that it must therefore be obtained from outside either as such from animal sources or from plants in the form of phytosterol.

There is some evidence that a deficiency of cholesterol occurs in pellagra. M. L. Koch and C. Voegtlin (1916) have shown that certain chemical changes are noticeable both in the brains of advanced cases of pellagra and of monkeys which have been fed for a considerable time on an insufficient and ill-balanced diet. These consist chiefly in a disturbance of the normal ratio between the various lipoids of the cerebrum, the cerebellum and the spinal cord, there being a marked deficiency in the cholesterol and sulphatid content of the hemispheres, in some cases a deficiency, in others an apparent increase in the cholesterol of the cord. The degeneration of the nerve fibres of certain tracts of the cord commonly seen in pellagra, is shown by these authors to be almost exactly reproduced in the experimental animals.

The fats of the sebaceous secretion are composed largely of cholesterol fats and the absence of this secretion in sufficient amount accounts very largely for the characteristic dryness and perhaps other cutaneous symptoms in pellagra.

The writer has not been able to find any statement as to the cholesterol content of the various vegetable foodstuffs. It is however improbable that the cholesterol content of the diets known to have been pellagrous (Table XX) can be less than that of diets 19 and 21, consisting mainly of rice, in which the fats are reduced to a minimum.

The above facts are brought forward not with the intention of suggesting that a deficiency of lipoids (or in particular cholesterol) is the cause of the

disease but that it may be a contributory factor at any rate in the production of some of the nervous and other lesions.

PROTEIN.

That a deficiency in the amount and quality of the food is of primary importance in producing the disease, has been pointed out by the majority of observers from Casal onwards; as also the reciprocal fact that a generous diet will cure the disease.

The evidence of the diets given in the table as well as that of the orphanage and asylum experiments recorded by Goldberger and his co-workers and Ridlon in America, prove this latter point. The fact that the disease affects especially the very poor is well recognised. Siler and Garrison (1914) show that of the cases reported 83 % were in a state of poverty, 15 % comfortably off, 2 % in affluent circumstances; with reference to the latter it is remarked that the presence of abundant food is no proof that it is eaten. Sandwith (1905) states that in some villages of lower Egypt which he visited in the year 1902, 62 % of the population appeared to be pellagrous; these villages were occupied by fellahin employed upon the State Domains Administration lands at a daily wage amounting to about three piasters ($7\frac{1}{2}d.$) daily, an amount that, even at that date, was not sufficient to supply a small family with sufficient food. He contrasts these villages with others in the same region occupied by more prosperous peasants in which only 15 % of the men appeared to be affected.

Sydenstricker (1915) states as an explanation of the increase of pellagra in the United States, that in those regions most affected, while wages had increased between the years 1900 and 1913 by 25 %, the cost of foodstuffs had risen 60 %, also (*l.c.* p. 19) that the increase in the price of the protein of the food had risen to an extent which was 60 % greater than in the case of carbohydrates and fats. In the Spartenburg Report (p. 7) it is stated that "the most striking defect is the absence of fresh meat, animal protein being supplied largely in the form of salt pork, especially bacon."

These few references are sufficient to show that while insufficient food has been generally recognised as the cause of pellagra, particular attention has been directed to a deficiency in the protein.

The series of diets shown in Table XX has been collected and the nutritive value estimated for the purpose of elucidating this relationship of protein deficiency to pellagra. That such a relationship actually exists and is probably the essential element in the etiology of the disease, appears to be clearly demonstrated by the figures in column 4 (giving the biological value of the protein in the different dietaries). It will be seen that these diets having a biological value in protein of 30 or less are all primarily pellagrous, while those with a biological value of above 37 are relatively non-pellagrous in cases in which the diet is fully sufficient to meet the energy requirements but apparently not so, as in 13 and 15, where the energy value is below the amount

required by the work (non-pellagrous, that is in regard to normal persons who have not previously shown symptoms of the disease).

None of these diets with the exception of 9 are such as to prevent the re-appearance of the disease in persons previously affected.

(*Note.* Since writing the above an experimental investigation has been carried out on a group of healthy men selected from among those receiving diet 12, in collaboration (1918) with Capt. H. E. Roaf. It was found that owing to the character of the bread and the mode of preparation of the rest of the rations the loss of protein in the intestine from non-absorption was 33 % in place of 25 % as estimated from the absorbability of food-stuffs composing the diet as given in the literature on the subject. The defective absorption of protein reduces the biological value to 33 and accounts for the considerable incidence of pellagra among these men. This diet is a good example of a diet on the border-line of protein sufficiency in which an insufficiency arose from the defective digestibility of the food as issued for consumption. It is not improbable that, if it were possible to examine certain other diets which appear to have been pellagrous but in which the protein value does not appear to be sufficiently low to explain this, a similar result might be obtained.)

ANIMAL PROTEIN.

With regard to the nature of the protein, it will be noted that animal protein is completely absent in diets 1, 2, 3 and 10 and small in amount in 7, 11, 12 and 14, animal protein is however completely absent in 19, 21 and 23 which, as far as any record to the contrary shows, are non-pellagrous.

Although it has been found to be impossible to rear growing animals—mammals—on a purely vegetarian diet (McCollum, *l.c.*) it is doubtful if this applies to the maintenance of full-grown animals. There is in fact no doubt that the majority of the poorer inhabitants of far eastern countries (especially where rice is the chief cereal) live on a purely vegetarian diet.

THE VALUE OF MAIZE.

The question as to the part played by maize in the causation of pellagra seems to be answered in the main by the low biological value of maize protein, one-third that of animal protein as found by Thomas. This is largely accounted for by the fact that 50 % of the protein of the endosperm is in the form of zein (Osborne and Mendel, 1914).

That pellagra is not due to maize as such is demonstrated by the fact that some of the diets (12, 13, 14 and 15) contain no maize product of any kind. The occurrence of 10 recorded cases in different asylums in Great Britain (Sambon, 1913) where maize products were not used, is evidence of this; as also the 15 cases among a rice-eating community in the Straits Settlements recorded by Sidney Sheppard (1912).

That it is impossible to maintain animals in a state of health on a diet in which zein forms the only protein was shown by Osborne and Mendel (1911). Sandwith (1913) appears to have been the first to suggest that the absence of tryptophane in zein is related to the pro-pellagrous character of maize. Further evidence in regard to the question of the bearing of the tryptophane deficiency on pellagra will be discussed below.

The low value of maize as a source of protein is however not entirely

due to the presence of a large percentage of zein (it must be remembered that other proteins are present, which contain at any rate a small percentage of tryptophane), but also to the fact that maize is more variable in its protein content than probably any other cereal. Mayrhoft (1912) gives the range of variation as from 5.5 to 14.8 % and the writer has himself analysed a sample of bread consisting chiefly of maize flour which contained nitrogen equivalent to only 4.8 % of protein having a biological value of no more than 1.4. It is clear that on the lowest computation it would be necessary for a man to eat daily no less than 2140 g. (4½ lb.) of such bread to obtain protein having a biological value of 30.

The better milled the maize flour, the less will be the protein content and the higher the proportion of zein to total protein. Osborne and Mendel (*l.c. supra*) find that zein forms 41.4 % of the protein of the whole kernel. This may explain the improvement in the prisoners referred to by Nightingale (*l.c.*) when their diet was changed from the highly milled maize to the roughly ground millet.

The comparative rarity of pellagra in rice-eating communities must be ascribed to the high ratio 1/1.1 of the protein as compared to that of other cereals, especially maize, the ratio of rice to maize being in this respect 3 : 1.

Sheppard (*l.c.*) describing 15 cases of pellagra in the Straits Settlements, rice being the chief food of these patients, remarks: "the patients are of the poorest class and further observation leads one to think that even rice, the cheapest food, was not always within their reach."

The value of the protein in diets 19 and 21, in which rice probably formed 90 % of the food, it will be seen, in spite of the low calorie value of 2240 and 2110 respectively, is higher than that of any of the pellagrous diets given. It is almost certain that although the first and probably the second of these two diets may have given rise to beri-beri, cases of pellagra did not occur; in regard to this point Greig (*l.c.*) gives a careful analysis of the symptoms seen in some hundreds of cases of epidemic dropsy traced to diets similar to 19, and in no case is any symptom described which might possibly have been the cutaneous manifestation of pellagra.

Sufficient has been said to support the view that pellagra is more closely related to a deficiency or inadequacy of the protein value of the diet than to any other dietetic cause.

SIGNIFICANCE OF INDICANURIA.

There are certain facts which seem to suggest that tryptophane may be the essential component of protein, the deficiency of which may be responsible for the disease. A deficiency of lysin may be of equal importance. Both these amino-acids are absent from zein and both are essential in an adequate protein. Osborne and Mendel (1916) show that a minimum amount of tryptophane and lysin are required for growth and nutritive equilibrium. (See also Chick and Hume, *l.c. supra*.)

The question of the proteins of maize has been already dealt with. Milk, the caseinogen of which contains 1·5 % of tryptophane and 5·8 % of lysin (Plimmer), appears to be generally regarded as the most efficient addition to the diet as an anti-pellagrous measure.

The gliadin of wheat, forming a little less than half the total protein, contains as much as 1 % of tryptophane but no lysin; other important amino-acids, phenylalanine, tryosine and histidin, are however deficient in the proteins of wheat as compared to those of milk. Such differences no doubt account for the differences in biological value.

Tryptophane has been shown by Hopkins and others previously referred to (a) to be an essential element in the food for maintenance and growth; (b) not to be built up in the animal organism, in this last respect resembling others of the higher amino-acids, at any rate those united to an aromatic or hetero-cyclic nucleus.

Tryptophane (indol-amino-propionic acid) is peculiar in that the protein molecule appears to contain no other amino-acid in which occurs the indol ring; it must therefore be present in sufficient amount as such in the protein of the food, whereas in the case of others, as for example arginin and histidin, the structure is sufficiently similar to allow of their mutually replacing each other in the food (Hopkins and Ackroyd, 1916).

The fact that tryptophane cannot be formed in the body but must be obtained from the products of the intestinal digestion of the proteins taken in the food, implies that the skatol and indol frequently found in the faeces and the derivative of indol "indican" found in the urine, must be formed at the expense of the tryptophane of the food protein.

That indol has its origin in the large intestine was shown by Ellinger and Gentzen (1904). Ellinger (quoted by Mackenzie Wallis, 1911) also shows that the injection of indol into the caecum is rapidly followed by the appearance of indican in the urine, and Assayama (1916) has demonstrated that alkaline solutions of tryptophane given by the mouth or injected into the large intestine greatly increase the indican of the urine, subcutaneous introduction of such solutions giving no result.

The importance of tryptophane in nutrition renders the loss from intestinal putrefaction a matter of considerable importance when the amount in the proteins ingested is minimal. Some amount of intestinal disturbance is commonly associated with pellagra and it has been frequently observed that pellagrins excrete excess of indican.

Marie (*l.c.* p. 218) states that he has almost constantly found indican in the urine. Ormsby and Slinger (1911) found a marked indican reaction in the urine of all cases examined. Myers and Fine (1914) found indican present in all the 13 cases examined, the daily excretion of this substance varying from a minimum of 21 mg. in one case to a maximum of 240 mg. in another. In April 1917 the writer examined 38 cases at the Tura Convict Prison, 3 of the men were in hospital, the remainder under supervision; the pellagrous

symptoms were no longer visible in a number of these. Of these men 3 showed indican in large amount, 21 in smaller quantities. Of 64 Egyptian medical students, said to be in good health, 7 only showed indican in the urine; 4 traces, 2 well marked, and 1 in large amount; the latter stated that he had recently suffered much from dyspepsia and diarrhoea. This examination was made in the hot weather of May. An examination made in the cold weather of the previous January gave a similar result. Hunter, Givens and Lewis (1916) find indican very generally present in considerable quantities in pellagrins and make the interesting observation that indol-acetic acid is present in large quantities inversely with the indican (see their tables, pp. 48, 49).

The following results (in percentages) were obtained recently in the examination of 152 apparently healthy Egyptian medical students made by the writer and Dr A. M. Mahmoud Bey (assistant in the Physiological Dept.):

None, 73.8; trace, 11.8; below $\frac{1}{16}$, 7.2; below $\frac{1}{4}$, 5.9; below 1, 1.3; above 1, 0.

My friend, Capt. H. E. Roaf, R.A.M.C. (T.), with whom I have been recently collaborating in the investigation of certain aspects of the pellagra question, gives me permission to quote the following figures:

Table XXII.

Amount of Indican in the Urine of Turkish Prisoners on Diet 12.

Healthy	None	Trace	Below $\frac{1}{16}$	Below $\frac{1}{4}$	Below 1	Above 1
%	64.4	24	9.6	2	0	0
Quiescent pellagra						
%	34.3	19	21.9	13.3	11.4	0
Active pellagra						
%	7	5.6	28.2	31	22.5	5.6

The fractions refer to dilutions of Fehling solution with distilled water. A solution of indigo-carmin may be used in place of the Fehling, one part of indigo-carmin in 5000 parts of water being almost exactly equal in tint to undiluted Fehling. From this it is possible to estimate roughly the amount of indican present in the urine, 200 mg. per litre of indigo-carmin corresponding to 238 mg. of indican (ratio of molecular weights).

Taking this figure as a basis of calculation, the amount of indican excreted daily by active pellagrins would be: 33.8 %, 15 mg. or less; 31 %, 15-60 mg.; 22.5 %, 60-238 mg.; and 5.6 %, more than 238 mg. per litre of urine. Jaffé gives 5 to 20 mg. as the daily excretion of indican in healthy men.

Borden (1907) finds the average output to be 12 milligrammes, 40 being rare in health. Hunter, Givens and Lewis (1916) place the normal upper limit of indican in the urine at 25 mg.

Stanford (1911) draws attention to the frequent presence of indican in the urine in melancholic and akinetic forms of insanity. Such types of insanity commonly result from pellagra. Mackenzie Wallis (1911) in the same journal finds an increase in indican in many forms of insanity, combined with which they find a marked diminution and abnormal variability in the creatinin as well as in the neutral sulphur of the urine. It is interesting to note that Myers and Fine find the same conditions in pellagra. Mackenzie Wallis considers these abnormalities as evidence of an altered state of cell-metabolism regarding the indican, on what grounds is not stated, as being of endogenous origin

and not as arising from intestinal fermentation. This author refers to the low physical condition of the peasants of Northern Italy as being possibly connected with the absence of tryptophane in the zein of the maize largely consumed in that region, without however mentioning pellagra.

It is difficult to avoid the impression that many of the cases of insanity investigated by these two authors in English Asylums, may have been pellagrous.

Myers and Fine (*l.c.*) show that in addition to indican, the other ethereal sulphates are increased to a corresponding extent. This indicates that the aromatic amino-acids are destroyed to a like extent with tryptophane and hence it may fairly be assumed that the indican of the urine represents the destruction of the whole protein molecule as far at any rate as many of its most essential components are concerned and that the indican may therefore be taken as a measure of the loss of a corresponding amount of protein to the body.

The molecular weight of tryptophane is 204; of indican, the potassium salt of indoxyl sulphonic acid, 251.

240 mg. of indican, the maximum¹ amount found by Myers and Fine (*vide supra*), would be derived from 195 mg. of tryptophane, an amount contained in 13 g. of caseinogen or 20 g. of the gliadin of wheat, representing about 400 g. of milk in the first case, or about 500 g. of wheaten bread in the second and very much more than this amount of maize bread. It is true that the glutelins and other proteins of bread contain some tryptophane; at the same time it will be readily seen that the indican of the urine may be an evidence of a very large loss of protein to the body through its bacterial destruction in the digestive tract.

Myers and Fine state that there is a great increase of indol and skatol in the faeces of pellagrins while the faeces of normal individuals contain neither of these substances.

The urinary indican represents therefore only a part of the loss of protein to the body.

A-CHLORHYDRIA.

Niles (1912) found in 64 cases of pellagra that the gastric HCl was absent in 18, diminished in 31, normal in 3, increased in 12; Myers and Fine find it absent or greatly diminished in all cases; related to this they form the conclusion that "the presence of much indican with high ethereal sulphate elimination and much indol and skatol in the faeces indicate peculiar bacterial conditions, probably high up in the intestine."

Hunter, Givens and Lewis (*l.c.*) find HCl absent in 52 %, deficient in 10 %. They state that a-chlorhydria, once present, is not recovered from in pellagrins, even when the other symptoms of the disease disappear. This fact, if true, has an important bearing on the appearance of the relapses so common in

¹ In 5.6 % of the cases examined by Roaf this amount was exceeded.

this disease and on the impossibility of curing cases of pellagra after they have passed beyond a certain stage.

The absence or diminution of HCl in the gastric juice has been noted by Moore and Roaf in many debilitating diseases, especially cancer, whether in such cases it was accompanied by indicanuria was not observed.

That such a condition would lead to the invasion of the intestine by an excessive number of micro-organisms is doubtless true; it would also lead to other effects tending to produce defective absorption of proteins in the small intestine thereby favouring their destruction by bacteria.

The absence of the HCl of the gastric juice or even its considerable diminution, would result almost certainly in a diminution of the pancreatic secretion¹ by the removal of the normal stimulus to the production of secretin. This would no doubt lead to incomplete digestion of protein and its passage onwards into the large intestine, the partially digested or undigested protein undergoing putrefaction either in the small intestine or after its passage into the large intestine with the production of useless waste products, manifested by the appearance of indican in the urine and a more or less important loss to the organism.

Myers and Fine (*l.c.*) examined the excreta of their patients and concluded that the nitrogen absorption in pellagrins was not markedly different from that of normal individuals; this may be true and yet the nitrogen may be absorbed in a useless form to the organism as pointed out above. Such substances as for example indol would be excreted in the urine, their nitrogen would not appear in the analyses of the faecal output but would be included in the presumed metabolised nitrogen of the urine. It is clear therefore that the determination of the total nitrogen excretion by the urine and faeces respectively gives in pellagrins a false picture of the assimilation of proteins.

(Note. In the experimental investigation of the nitrogen assimilation from diet 12 (see p. 28) a group of 5 pellagrins was examined, the conditions being the same as those for a similar group of healthy men. The loss of protein by the intestine was 33 % in the healthy, 35 % in the pellagrous group, a comparatively small difference.

The urine of the pellagrins contained however in all cases some indican, this substance being absent in the case of the healthy men.

If the loss of protein, which a given amount of indican represent, be considered, it will be obvious that in some cases, at any rate among the men examined, the total loss of protein must have been much in excess of the 35 % actually shown by the estimation of the faecal nitrogen.)

The writer has attempted to show that, measured by the indican of the urine, the protein destroyed in the intestine by bacterial action may amount to a considerable part of the total nitrogenous intake.

If this be so it will be easily understood that (a) persons in the general community suffering from indicanuria would, if reduced to a protein intake near the minimum requirement for normal people, be actually living under

¹ Evidence is given of this by the assimilation experiment referred to on p. 28. It was found that in the pellagrous group examined, 28 % of the fat intake was un-absorbed (Soxhlet extract of faeces) as compared with 19 % in the healthy group.

conditions of protein starvation, and that they would be necessarily more liable to develop a disease due to protein deficiency than other persons. Thus even on what might be considered a quite adequate dietary for normal individuals, persons with the very marked indicanuria not unfrequently seen in apparent health might suffer from protein deficiency. It is in fact not an improbable supposition that the indicanuria so generally present in pellagra is not entirely a resultant symptom of the disease but that those members of the population who suffer from indicanuria and the conditions leading to it are unable to maintain their nitrogen equilibrium on a protein intake fully sufficient for quite normal people and are therefore predisposed to pellagra.

(b) If there existed a chronic condition of deficient gastric HCl or defective pancreatic digestion accompanied by excessive bacterial fermentation apart from, or acquired as the result of an attack of pellagra, that persons so affected would be in a condition in which they would require more protein, possibly much more, to provide them with their minimum requirements.

It would not be difficult under such circumstances to understand the fact that recovered pellagrins tend constantly to relapse if living under similar conditions to the poorer population generally, or even occasionally when provided with what would ordinarily be considered a very liberal diet.

At the Abassia Asylum, where relapses are not uncommon, an example of such a case is seen. A few relapses occurred at the Refugee Camp even after the antipellagrous diet was introduced in February 1917.

The question of indicanuria has been discussed at some length as it appears to have a bearing on the part played by protein deficiency in the causation of the disease, the significance of which has not been sufficiently appreciated.

INDIVIDUAL VARIATIONS.

It is not intended to suggest that only persons with indicanuria or digestive disturbances develop pellagra; this is certainly not the case. Among the poorer classes of a community living on barely sufficient food, there must be many individuals whose normal protein requirements, apart from the above causes, exceed the average, and probably others who can maintain health on a minimum of protein considerably below the average.

Earlier in this paper (p. 13) the writer has attempted to illustrate this normal variability by an approximate estimate of the differences in the minimum protein value required by the five subjects of Chittenden's experiment, the requirement for the maintenance of nitrogen equilibrium varied from rather below 30 to about 40 g. (See also Sherman, *l.c. supra.*)

If a population be imagined of this character living on a diet having a value in protein of 37, it is obvious that while the majority would be obtaining sufficient for their normal requirements, a certain percentage would be suffering from chronic protein deficiency. Border-line diets such as this will be found among those presented and it will be seen that the incidence of pellagra on the communities affected accords well with the explanation offered.

INFLUENCE OF WORK.

That the calorie value of the diet has no direct relationship to pellagra appears from an inspection of the table of diets (Table XX and Chart III).

It will be seen that certain diets (*e.g.* No. 10) of a relatively high calorie value, are pellagrous, while others (No. 22 and 25) have calorie values of bare subsistence values but are not known to have been pellagrous.

There are however in the table three diets in which the indirect effect of a deficient energy intake appears, namely 13, 14 and 15. (For statistical details upon which the conclusion as to the influence of these three diets is based, see Part I, pp. 28-33.) The explanation of this indirect effect of labour must be sought in the well-established fact that to maintain the nitrogen equilibrium on a diet which is below the energy requirements, an increased supply of protein is necessary (ref. Argutinsky and Krummacker, 1890; also Cathcart, *l.c.* p. 111); and also that work, especially if severe, raises the protein requirements apart from the use of protein in the body as a source of energy (ref. Zuntz and Schumberg, Wolpert and Broden, 1901; Dunlop, Noël Paton, Stockman and Maccadam, 1897; Melville with J. S. Haldane, M. S. Pembery and others, 1913).

It is clear, if the nitrogen metabolism is influenced by labour or by work carried out under unfavourable conditions, that on such diets as those discussed with a narrow margin of sufficiency in protein, an increased incidence of pellagra will accompany an increase in the energy requirements if these are not fully met by the energy intake.

GENERAL REMARKS.

A considerable number of cases of pellagra are recorded in the medical literature of the British Isles an explanation of which, on the lines suggested in this paper, appears difficult; all such cases will remain obscure until, in addition to the observation of clinical details as regards symptoms, some attempt is made to apply modern methods of bio-chemical investigation to their elucidation, especially in regard to the true protein intake and the coefficient of protein assimilation.

The most common type of such cases is that described by Hammond (1913) in which a long period of intestinal trouble preceded the appearance of the cutaneous symptoms; a number of similar cases are described.

It can scarcely be doubted that secondary protein deficiency must have existed resulting from defective powers of assimilation and destruction of protein in the intestine by bacterial action.

(*Note.* It has been pointed out to the writer as a matter for surprise that, supposing pellagra to be due to a deficiency of protein, symptoms of the disease should not have been noticed in sprue in which a very chronic form of enteritis not unlike that seen in pellagra occurs, often leading to great emaciation. If however Bahr's monograph (1915) on the subject be consulted, it will be seen that there are important bio-chemical differences between the two diseases. In sprue the coefficient of protein assimilation is high, indicanuria is not a marked feature and the

gastric HCl tends to be increased rather than diminished; it appears therefore that secondary protein deficiency such as may lead to pellagra, is unlikely to occur in this disease.)

The writer does not propose to discuss any of the non-nutritive theories of the disease from the alleged effects of the presence of an excess of alumina or silica in the drinking water to infective agents, bacterial, protozoal or other.

Anyone reading the extensive literature of the subject must be impressed by the contradictory nature of the evidence and the insufficiency of the explanation offered, and, with regard to the infective theories, the completely negative results. Reference may be made in this connection to the work of Goldberger and Anderson (1911), Lavinder, Francis, Grimm and Lorenz (1914), O. S. Hillman (1914), W. J. McNeal (1914), Francis (1917) and White (1919).

In bringing to an end this survey of the part played by protein deficiency in the production of pellagra, it is clear that much useful work remains to be done. This is especially true of the biochemical problems involved and of the minute anatomy of the various tissues of the body particularly of the digestive system. A great advance has been made in the latter region of investigation by Sundwall (1917) who has demonstrated the very important fact, by comparison of the tissues of pellagrins with those of underfed or improperly fed animals, that all those changes seen in pellagra in the nervous system and elsewhere can be ascribed mainly to malnutrition. Almost of necessity the material examined came from the bodies of persons dying of the disease in its cachectic state. It would be of the greatest interest to observe the conditions of the tissues of the digestive tract before the condition has reached the stage when actual destruction of tissues has concealed the earlier atrophic changes.

It appears to the writer that such investigations would throw light on the course of events induced by protein deficiency and might possibly increase the existing knowledge of protein metabolism in general.

CONCLUSIONS.

The following conclusions may be drawn from the study of the pellagrous and non-pellagrous diets brought together and the biochemical and clinical information collected.

1. Pellagra is the ultimate result of a deficient supply of protein.
2. The sufficiency or insufficiency must be judged of by the biological value of the protein, estimated on the amount available for assimilation and not on the gross protein: 40 may be taken as the minimum safe value for this factor. Below this cases are likely to occur in the affected community: owing however to the great normal variations in the minimum protein requirement, many individuals, the biological value of whose daily protein intake is as low as 20, will escape the disease, while it is possible that some, with a value above 40, may become affected.

3. The deficiency of protein may be:

(a) *Primary*, in which the supply is insufficient for the individual requirement or, when, owing to the indigestible character of the food, a somewhat restricted supply cannot be utilised to the normal extent.

(b) *Secondary*, in which owing to digestive disturbances, or other causes, the supply of protein cannot be assimilated.

4. In accordance with conclusion 3, three types of pellagra may be distinguished etiologically:

(a) The common type seen in poverty or deficient food from other causes.

(b) Relapse cases in which, owing to permanent defects—the result of a previous attack of pellagra—the protein supply is inefficiently utilised.

(c) Cases in which a disease of the digestive organs due to other causes than insufficient feeding, leads to defective utilisation of the protein intake.

5. That indicanuria is an important indication of the loss of protein in the intestine, the amount present being sometimes sufficient to account for the loss to the body of a large proportion of the protein intake.

6. Indicanuria is closely related to the deficiency of gastric hydrochloric acid. This is due to two causes:

(a) The resulting invasion of the intestine with bacteria.

(b) The absence of the normal stimulus to the secretion of pancreatic juice, whereby less protein is completely digested and more is lost in the large intestine.

7. Labour raises the level of protein requirement, an effect which is increased greatly by a deficient energy supply. Labour is therefore a factor in the causation of pellagra in a community whose protein supply is on the border-line between sufficiency and insufficiency.

8. There is evidence which seems to suggest that a deficiency of cholesterol may be related to some of the symptoms.

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A NEW METHOD FOR THE ISOLATION OF ORGANISMS FROM FAECES AND SPUTUM, WITH SOME OBSERVATIONS ON HAEMOLYTIC STREPTOCOCCI IN FAECES OBTAINED BY THIS METHOD.

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PROFESSOR L. S. DUDGEON, when consulting bacteriologist to the Forces in the Balkans during the late war, had tried the effect of drying the stools of suspected dysentery patients on some porous material so as to leave only the mucus. In bacillary cases he found that a more abundant growth of dysentery bacilli was obtained from the dried mucus than from the untreated specimen, so that the drying had no harmful effect on the organisms. He used for this purpose unglazed red tiles such as were to be found locally, and suggested that the method should be given a more thorough trial with a more satisfactory type of tile made in this country. With this view the following experiments were made.

DUDGEON'S METHOD.

A portion of the faeces, about a small teaspoonful, is evenly spread over a porous tile resting on an asbestos mat and allowed to dry for from 30 minutes to two hours, at room temperature, according to the amount of water or mucus in the specimen. When the faeces are dried to a stiff paste, this is scraped off and evenly spread on a second tile and allowed to dry for a further one to two hours or until it has become a fine dry powder when scraped off. A quantity of this dry powder is transferred to a plate, and evenly spread over the surface with a glass spreader. If the faeces are liquid, as much as possible should be poured on the first tile short of letting it run over the edge. By using two tiles one after the other, it will be found that however liquid the faeces are, or however much mucus is present, complete drying can be obtained. It is important, in order to get the best results, that the material should be completely dried on the second tile before plating, and when scraped off, be in the form of a sand-like powder, with no trace of stickiness.

By this method excellent separation of individual colonies is obtained over the whole surface of the plate with no tendency to run together so that suspicious colonies can be picked off with ease. It is quick, and since it is equally

¹ The expenses of this investigation were defrayed by a special grant from Mr Louis Oppenheimer, to whom I am greatly indebted.

applicable to any infection, is most economical with media since different methods do not have to be adopted according to the nature of the organism suspected of being present. Using other methods than the tile method on an erroneous supposition of the infecting organism, the latter may be easily missed, as will be shown later.

By this method of drying on tiles, it is extremely easy to isolate small portions of mucus, which would be missed if the solid stool were examined, and thus is a most suitable procedure in enabling one to examine likely portions for *Entamoeba histolytica* cysts. Naturally in this instance complete drying is not resorted to, but just sufficient to produce a stiff paste in which pieces of mucus are easily seen. The same procedure can be followed also in searching for the ova of parasitic worms.

The method is also very suitable in plating from sputum. Excellent separation of individual colonies is obtained and a delicate organism such as the pneumococcus can be recovered in abundance when present. The same procedure is adopted as with faeces. A thick layer of sputum is spread on a tile and allowed to dry to complete dryness. During the process of drying both with faeces and sputum, the tiles should be covered over with a bell jar to protect them from contamination from dust, etc. The dried sputum is scraped off and spread over some suitable plate. The dried sputum when scraped off will be found to be a light, flaky, very fine powder, in contrast to dried faeces from which the powder is like sand and much heavier. For this reason, because of the extreme lightness of dried sputum, it is advisable when scraping it off the tile, to work behind a screen. A suitable screen can be made from an old half plate size photographic plate fixed on a piece of wood as a base, a deep groove being cut in the wood block at an angle in which the plate is fixed and thus inclined slightly inwards.

The tiles used are white unglazed porcelain tiles about 6 inches square. For use each large tile is divided into four pieces which gives a more convenient size to spread the material on. When required for use they are sterilized by heat, an electric hot plate is very convenient, but this can be done equally well by placing them on an asbestos cooking mat supported on an iron tripod and heating them with a Bunsen burner. Such asbestos cooking mats can be obtained from any ironmonger or stores. When cooled the tiles are ready for immediate use. For spreading the faeces and for scraping off the dried residue an old knife, especially kept for the purpose, is very convenient, since it can be easily sterilized by heating to redness.

The spreading of the material used, and the scraping off should be done with the tiles on an asbestos mat, as it is difficult to be certain that no powder has fallen off the tile, while if an asbestos mat is used, the whole mat can be sterilized in the flame immediately after use. When the tiles have been used, it is convenient to drop them into a bucket of some suitable disinfectant. At intervals they are collected, and before being put into use again, burnt dry by heating them over a small crucible furnace. It will be found that with tiles

that have been used several times, drying of the material used is somewhat slower.

The method of drying on tiles was used in the following series of examinations of stools of patients known to be suffering from an infection of the Typhoid group. The large number of negative results is due to the fact that before discharge three consecutive negative examinations were made. In addition to the dry method a loopful of faeces was added to a series of 5 c.c. peptone water tubes to which 0.04, 0.08, 0.12, 0.22 of a c.c. of a 1/10,000 solution of brilliant green had been added. One loopful of the top layer of fluid from each of these tubes was plated after they had been incubated for four hours at 37° C. One plate was made from the dried faeces. This procedure was followed for every stool examined. MacConkey's neutral red bile salt lactose agar was used for plating on. It will be seen that incubation of the cultures in brilliant green peptone water was for 44 hours, and not 24 hours as recommended by Browning (1919), or 7 to 12 hours as practised by Mackie (1919).

The following results were obtained:

Total number of examinations	64
Negative results by both methods	42
Total positive results by dry method	19
Positive from peptone water brilliant green; negative by the dry method	2

Amongst the 19 positive results by the dry method are included ten examinations in which positive results were also obtained from brilliant green peptone water, leaving nine examinations in which positive results were obtained by the dry method only. These 19 positive results by the dry method are additionally favourable to this method, as even when positive results were obtained by the alternative method, the former means a considerable saving of media. This is not so important in the few cases examined in this instance, but would be so when dealing with large numbers of cases.

These 64 examinations were made from 18 cases of "Enterica," 15 cases of infection by *Bacillus typhosus* and two by *B. paratyphosus* B. By the term positive result is meant that one of these organisms was isolated from the faeces, the cultural reactions of the organism being confirmed by agglutination of a 24 hours culture on agar.

All cases were examined once a week so that in some cases the infecting organism was isolated on more than one occasion from the faeces, while there were four cases in which no bacilli of the "Enterica" group were isolated on any occasion by either method. Three of these latter cases were infections by *B. typhosus*. All contracted Typhoid while under observation, from the fourth case to be mentioned later. *B. typhosus* was recovered by blood culture in all three. They all had a very mild attack of typhoid fever, the duration of the fever was so short and they were so mildly ill, that but for the fact that the diagnosis was made bacteriologically, it is improbable that Typhoid would

have been suspected, clinically they were so atypical, though this difficulty is denied by Garrow (1920). One of the three only had fever for four days with sudden onset of backache and pains in the limbs, and resembled influenza more than anything. The last case in which no typhoid bacilli were found in the faeces, was a case of cystitis from which *B. typhosus* was obtained in pure culture from the urine.

Amongst the already mentioned cases was one of some interest which illustrates very well the value of the dry method. The patient was suffering from typhoid fever from whom on the first occasion on which the stools were examined, typhoid bacilli were isolated by the dry method and also from peptone water brilliant green. At the second examination *B. dysenteriae* Shiga, confirmed culturally and serologically, was isolated by the dry method only, and no typhoid bacilli by either method. It appeared that the patient had been a soldier and had contracted dysentery in the East during the war. He must have been still harbouring dysentery bacilli which the attack of typhoid fever had aggravated. There was no reason to suspect dysentery in this case, as he was a known case of typhoid, so that except by the dry method, it is doubtful if they would have been isolated.

In addition to the above cases, one case of Flexner dysentery was examined. In this case, in addition to the dry method, a loopful of faeces was inoculated into a broth tube, incubated for one hour at 37° C. and a plate spread with one loopful from this tube. The following results were obtained from this patient.

Total examinations made	5
Negative results by both methods ...	3
Positive results by dry method only	1
Positive result from broth only ...	1

Since cases of dysentery are comparatively rare in England, the following experiments were made, employing normal faeces to which a small amount of a pure culture of *B. Flexner* (Gallipoli) Dudgeon (1919) was added. The normal faeces were made into a thick emulsion with saline. Two c.c. of this emulsion were employed to which was added one drop of an emulsion of the Flexner bacillus prepared by adding 2 c.c. of saline to a 24 hours' growth on an agar slope.

The resulting mixture of normal faeces and dysentery bacilli was plated by the dry method and also from a broth tube inoculated with a loopful of the mixture and incubated for one hour at 37° C.

The following results were obtained:

Total examinations	23
Negative by both methods	8
Total positives by dry method	13
Positive from broth negative by dry method	2

Of these 13 positives by the dry method, positive results were also obtained from broth in four instances, leaving nine examinations in which the dysentery bacillus was isolated in these artificial mixtures by the dry method only.

The total figures for all examinations both from patients with a bowel infection from the enteric or dysentery bacilli, and the artificial mixtures, is as follows:

Total number of examinations	91
Negative results by both dry method and alternative method used	53
Total positive results by dry method	33
Total positive results by alternative method, <i>i.e.</i> either peptone water brilliant green for the enteric group, or from broth for dysentery infections with negative results from dry method	5

Of these 33 positive results by the dry method, positive results were also obtained in 14 instances by one of the alternative methods, according to the nature of the infection, 19 examinations in which the dry method was successful and the alternative method unsuccessful.

OBSERVATIONS ON STREPTOCOCCI IN FAECES ESPECIALLY WITH REGARD TO HAEMOLYTIC VARIETIES.

Attention was drawn to the ease of isolating streptococci from the faeces by the dry method from the fact that, in the series of examinations of typhoid patients already mentioned, streptococci were isolated on MacConkey plates in great abundance on 15 occasions. In nine cases the patient was a known case of typhoid, in two dysentery, one was a case of coeliac disease, one of sprue, and one a case of diarrhoea of one year's duration in a child of four.

For this reason further examinations were made on a medium more suitable for the isolation of streptococci. Since blood agar would also show the presence of haemolytic colonies, this medium was used throughout. In this manner streptococci can be grown in abundance from any sample of faeces whether from normal healthy subjects or from patients with intestinal disease. In the course of examining the stools of 60 patients, 39 of which were normal healthy people, using the dry method and plating on blood agar, numerous colonies of streptococci were found in 58. In this series haemolytic colonies of streptococci were frequently observed, in view of this fact, the streptococci isolated by this method were investigated further.

Haemolytic streptococci in faeces do not appear to have been very frequently observed. Davis (1920) investigated the fate of haemolytic streptococci in the intestinal canal. Plating from broth on blood agar, he examined the stools of normal rabbits and found no haemolytic streptococci. He then fed the rabbits with haemolytic streptococci and found that he could recover the same type of organism from the faeces in the day immediately after the first day of feeding, but that they quickly disappeared and were not recovered again, however long the feeding experiments were continued. He next examined human faeces from 53 cases of all varieties, but found no haemolytic streptococci, although Pilot and Davis (1919) had shown that these organisms were present in 100 per cent. of cases in the crypts of the tonsils. He also examined the faeces of four scarlet fever cases but found no haemolytic streptococci.

Oppenheim (1920) examined 55 stools from 15 patients from which 323 strains of streptococci were isolated. He inoculated blood agar plates directly from the faeces. All streptococci isolated were tested as regards their fermentation properties, with the following results:

Percentage fermenting	glucose	...	100
..	lactose	...	97
..	salicin	...	97
..	inulin	..	1
..	mannite	..	76

Amongst these 323 strains he found five that had haemolytic properties.

Moody and Irons (1920) examined the stools of patients suffering from scarlet fever. They also examined the throats of all their cases and found haemolytic streptococci in every case. They examined 309 stools from 85 patients. They employed saline emulsions of the faeces plating this on blood agar, *i.e.* agar + 10 per cent. of goat's blood. They found streptococci present in the faeces of 26 patients, and from these 26 cases isolated 22 strains of haemolytic streptococci. All these haemolytic organisms fermented lactose and salicin, but not mannite or inulin.

Dible (1921) studied 152 strains of streptococci all isolated from faeces. For isolation he employed either direct plating on agar plates, direct plating on MacConkey's bile salt lactose agar, or by methods advocated by Thiercelin (1902) for the isolation of streptococci from faeces, chiefly anaerobic cultivation in broth followed by plating on agar. He studied the fermentation properties of all strains using Hiss' serum water medium plus the addition of various "sugars." All strains were tested for haemolysis. For this purpose he used an equal volume of the culture of the organism to be tested and of 10 per cent. washed rabbit's corpuscles. The culture medium used was serum one part, broth four parts. The mixture of serum broth and rabbit's corpuscles was incubated for 2½ hours at 37° C., placed in the cool overnight and read next morning. He found that the streptococci dealt with were of two types culturally, one forming minute pin-point colonies on agar, the other much coarser colonies with a tendency to coalesce. Further the coarse colonies were usually diplococci on examination, whereas the fine colonies showed chain formation. In differentiating streptococci from enterococci he tested the statement of Houston and M'Cloy (1916) that their enterococcus would withstand exposure to heat and tested all strains for their ability to withstand exposure to 60° C. for as long as 30 minutes. He finds an intimate relation between, on the one hand, heat resistance, diplococcal form, ability to ferment mannite and coarse colony formation, and on the other, sensitiveness to heat, failure to ferment mannite, a tendency to form chains with fine colony formation. He considers the typical enterococcus to be diplococcal in form with the above characteristics and the following reactions on sugars.

Type	...	Litmus milk	Lactose	Inulin	Salicin	Mannite	Dulcitol
Variation I	...	+	+	-	+	+	-
" II	...	+	+	-	+	-	-
" III	...	+	-	-	+	-	-

In the series of organisms examined, he only found two haemolytic organisms, both forming long chains and both non-mannite fermenters, and non-resistant to heat. In the total series 85 strains were heat resistant, 52 heat sensitive.

Percentage fermenting lactose	...	78
" " salicin	...	93
" " inulin	...	17
" " mannite	...	53
" clotting litmus milk	...	72

Method. In the following series of examinations faeces were dried by Dudgeon's tile method in every instance. They were plated on blood agar made by adding 1 c.c. of human oxalated blood to 15 c.c. of melted agar at a temperature of about 50° C.

Haemolysis was noted on the plate and all strains were further examined for haemolysis by inoculating a 5 c.c. peptone water tube to which 0.1 c.c. of centrifugalized human red cells were added and well shaken to ensure a uniform suspension. These tubes were incubated at 37° C. overnight and read next morning. The cultural characteristics of all streptococci were examined with results noted in the table. The media employed were Lemco broth containing 1 per cent. of the various "sugars" used. All tubes were incubated for five days at 37° C.

Only one colony was examined from each plate, unless haemolytic colonies were present, when one of each was examined, namely, one haemolytic and one non-haemolytic. If haemolytic colonies were present they were never present in abundance, two or three per plate being the maximum, more often only one. The haemolysis on a blood agar plate was of two varieties, (i) a colony with a small diffuse somewhat opaque zone of haemolysis with a greenish colouration, and (ii) a sharply defined clear transparent completely haemolysed colourless zone 2-4 millimetres in diameter as defined by Brown (1919). The first variety never produced haemolysis in peptone water red cells, the second always did.

In the subjoined table the signs used have the following significance:

Against the sugar tubes	+	means production of acid.
" " "	-	" no change.
" Litmus milk	+1	" acid only.
" " "	+2	" acid plus clotting.
" Resistance to heat	+	" resistance to exposure of 30 minutes at 60° C.
" " "	-	" killed by exposure to 60° C. for 30 minutes.

Dible's observations on heat resistance were not seen until the majority of these results were completed, so that only a few organisms have been tested for this property. The method employed was to place a 24 hours' broth culture of the organism to be tested in the water bath for 30 minutes at 60° C. The tube was then stood at room temperature for one hour when three drops were inoculated on an agar slope and incubated for 24 hours at 37° C.

From a consideration of the table it will be seen that the diplococcal type is in the majority, few long chained streptococci being met with. In all 52

No	Nature of case	Haemolysis on blood agar plate	Haemolysis in red cells peptone water	Morphology	Lactose	Dextrose	Mannite	Dulcife	Inulin	Salicin	Starch	Litmus milk	Character of colony on agar	Resistance to heat
1	Rheumatoid arthritis	+	+	Diplococci and short chains	-	+	-	-	-	+	-	+2	Fine	...
2	Myelaemia	+	+	"	+	+	-	-	-	+	-	+1	"	...
3	Diarrhoea in infant	+	+	"	+	+	-	-	-	+	+	+2	"	...
4	"	-	-	"	+	+	-	-	-	+	+	+2	"	...
5	Typhoid	-	-	"	+	+	-	-	+	+	+	+2	"	...
6	"	+	+	"	+	+	-	-	+	+	+	+2	Coarse	...
7	"	-	-	Diplococci chains of 8	+	+	-	-	-	+	+	+2	Fine	...
8	Melaena	+	+	"	+	+	+	+	+	+	+	+1	"	...
9	"	-	-	Diplococcus	+	+	+	-	-	+	+	+2	Coarse	...
10	Colitis	+	+	"	+	+	-	-	-	+	-	+2	"	...
11	"	-	-	"	+	+	+	-	-	+	-	+2	"	...
12	Amoebic dysentery	-	-	"	+	+	-	-	-	+	-	+1	Fine	...
13	"	+	-	Diplococci long chains	+	+	-	-	-	+	-	+2	"	...
14	Melaena	-	-	Diplococci chains of 8	+	+	-	-	-	-	-	+1	"	...
15	Sprue	-	-	Diplococcus	+	+	+	+	+	+	+	+2	"	...
16	"	-	-	"	-	+	-	-	-	+	-	+2	"	...
17	"	+	+	Diplococci chains of 8	+	+	-	-	-	+	-	+2	"	...
18	Normal	-	-	Diplococcus	+	+	-	-	-	+	-	+2	"	...
19	"	-	-	"	-	+	-	-	-	+	-	+2	"	...
20	"	-	-	Diplococci	-	+	-	-	-	-	-	+2	"	...
21	"	-	-	"	+	+	-	-	-	-	-	+2	"	...
22	"	-	-	"	-	+	-	-	-	+	-	+2	Coarse	...
23	"	-	-	Diplococci chains of 4	-	+	-	-	-	+	-	+2	"	...
24	"	-	-	Diplococci	+	+	+	-	-	+	-	+2	"	...
25	"	-	-	"	+	+	+	-	-	+	-	+2	"	...
26	"	-	-	"	+	+	+	-	-	+	-	+2	"	...
27	"	-	-	"	+	+	+	-	-	+	-	+2	"	...
28	"	-	-	"	+	+	+	+	+	+	+	+2	Fine	...
29	"	-	-	"	+	+	+	-	-	+	-	+1	"	...
30	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
31	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
32	"	+	+	"	+	+	+	-	-	+	-	+2	Coarse	+
33	"	-	-	Diplococci chains of 6-8	+	+	-	-	-	+	-	+2	Fine	+
34	"	-	-	Diplococci	+	+	-	-	-	+	-	+2	"	...
35	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
36	"	+	+	Diplococci chains of 10	+	+	-	-	-	+	-	+2	"	+
37	"	+	+	Diplococci	+	+	+	-	-	+	-	+2	"	+
38	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
39	"	-	-	"	+	+	+	-	-	+	-	+2	"	...
40	"	+	+	Diplococci chains of 20	-	+	-	-	-	-	-	-	"	...
41	"	-	-	Diplococci	+	+	+	-	-	+	-	+2	"	...
42	"	-	-	"	+	+	-	-	-	+	-	+2	"	...
43	"	-	-	Diplococci chains of 6	+	+	+	+	-	+	-	+2	Coarse	...
44	"	-	-	Diplococci	+	+	+	-	-	+	-	+2	Fine	+
45	"	-	-	"	+	+	-	-	-	+	-	+2	"	-
46	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
47	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
48	"	-	-	"	+	+	+	-	-	+	-	+2	"	+
49	Pernicious anaemia	-	-	"	+	+	-	-	-	+	-	+2	"	+
50	Normal	+	+	"	+	+	-	-	-	+	-	+2	"	+
51	Pernicious anaemia	-	-	"	+	+	-	-	-	+	-	+2	"	-
52	Normal	-	-	Diplococci chains of 4-6	+	+	-	-	-	+	-	+1	"	-

strains were examined, 13 being haemolytic colonies on blood agar, 11 producing haemolysis of red cells in peptone water. The same two types of colonies were observed as mentioned by Dible, many of the diplococci morphologically could not be distinguished from pneumococci.

In the eleven strains that produced haemolysis in peptone water plus red cells, four were of the long chained variety, seven were diplococci. Four fermented mannite, seven did not.

Briefly the cultural characteristics of all strains examined were as follows:

(1) A group fermenting dextrose, lactose, mannite, salicin, and not dulcitate, inulin or starch. 19 in number.

(2) A group fermenting dextrose, lactose, salicin, and not mannite, dulcitate, inulin, starch. 13 in number.

Lactose was fermented by 45 strains or 86.5 per cent of all						
Dextrose	52	..	100
Mannite	24	..	46.1
Dulcitate	4	..	7.7
Inulin	5	..	9.6
Salicin	48	..	92.3
Starch	9	..	17.3

All with one exception, No. 40, acidified litmus milk, acidification and clot being produced by 45.

The term normal case in the table includes a variety of patients, the majority being cases of hernia in young adults, or patients admitted for the surgical treatment of deflected nasal septum, and a few cases for removal of tonsils, in which at the time of examination there was no obvious sign of tonsillitis.

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STUDIES UPON THE TOXICITY OF PUTRID FOOD.

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(A Report to the Canned Food Committee of the Department of Scientific and Industrial Research, Food Investigation Board.)

IN nearly every case where canned meat or fish is condemned as unfit for human consumption, the action is based upon the physical features of the unopened tin, which are held to indicate decomposition of the contents. No studies of canned foods would therefore be complete without some detailed consideration as to the real harmfulness of such rejected foodstuffs.

In view of the large quantity of tainted and unsound food rejected and destroyed annually, it is surprising that so few data exist as to the precise nature of the harmful substances in such food so that scientific reasons can be given to justify so extensive and widespread an administrative practice.

Existing evidence is both meagre and unreliable. Three fallacies vitiate nearly all the data we possess and render unreliable the conclusions drawn from the recorded facts.

The first of these fallacies is that the alleged harmfulness of meat or other foodstuff in a condition of incipient putrefaction has usually been confused with poisoning by food infected with specifically harmful bacteria. This is clearly brought out when the bacteriology of outbreaks of food poisoning is studied. At one time such outbreaks were widely and indiscriminately ascribed to changes in the food of the nature of putrefaction. More careful investigation has shown that the vast majority of such outbreaks are due to infection of the food by certain specific and highly pathogenic bacteria which are incapable of initiating putrefactive changes.

When outbreaks with this etiology are removed from the list it is found that those for which a definite putrefactive origin can be adduced are reduced to a vanishing point, and it is not too strong an inference to draw from the recorded outbreaks that no extensive outbreaks of food poisoning have ever been shown to be due to the consumption of food in a putrefactive condition, unaccompanied by a definite specific bacterial infection or the presence of toxins produced by such specific bacilli. It is not possible to exclude entirely a putrefactive origin in these cases in which individuals, or even small groups of individuals, have suffered, because the scientific records are so meagre, but diligent search has not revealed a single case in which the evidence proved that the attack was due to putrefaction uncomplicated by pathogenic non-putrefactive bacteria.

The second fallacy is that the chemical studies upon which the theory of the harmfulness of food in a condition of putrefaction has been built up rest upon a study of the end products of putrefaction and not upon an investigation of the toxicity of such foods in stages of putrefaction when there is a possibility of their being consumed. The whole ptomaine theory, built as it is upon such foundations, is entirely unsound.

The remaining fallacy is that nearly the whole of the evidence as to the alleged toxicity of the products of protein decomposition is based upon experiments upon animals in which the method of introduction is by injection and not by feeding. The introduction of the products of decomposition, alien as they are to the animal economy, directly into the tissues might be expected to produce intoxication which would not occur if the product had been introduced by the mouth and been subject to the action of the digestive juices. It is well known that there are a number of substances such as snake venoms and products of pathogenic bacteria, which are nearly harmless by the mouth but intensely toxic when introduced under the skin.

In view of the remarkably scanty evidence upon which the theory of the harmfulness of incipiently putrefactive food has been built up, it seemed advisable to test directly the harmfulness of food in the early stages of putrefaction, and the following experiments were therefore carried out as a small contribution to this difficult subject.

EXPERIMENTAL INVESTIGATIONS.

In the majority of the following experiments young kittens were selected as being the most suitable. They are more akin to man in the physiology of their digestive system than the usual laboratory animals, while the fact that they vomit readily is an important advantage in work of this character.

Owing to the work being carried out in association with other investigations, inability to obtain suitable animals just when wanted, etc., the actual experiments, although few in numbers, were spread over several years. The first ten experiments were carried out by me alone in 1918. Experiment 11 (in 1919) with the assistance of Mr R. B. Calder and Experiments 12 and 13 (in 1920) with the help of Mr Hunwicke.

Exp. 1. Raw meat was taken and kept at laboratory room temperature for three days when it became very offensive and putrid. Washings from the pulped meat strained through muslin were mixed with the rabbit's food and fed to the animal on three consecutive days. The animal showed no loss of weight and no ill effects of any kind.

A second rabbit was injected subcutaneously with 1 c.c. of the washings. The animal showed no obtrusive symptoms for the first two days, was noticeably weak and ill on the third day and died on the fourth day after the injection. A post-mortem revealed no gross lesions, and cultivations from the spleen and liver (both aerobic and anaerobic) and from the heart-blood (aerobic only) were all negative. There was no inflammation at the site of inoculation.

Although no bacteria were isolated, the length of time suggests bacterial infection from the putrid emulsion.

This experiment is of interest as an illustration of the fundamental differences between introduction by the mouth and under the skin.

Exp. 2. A young kitten, weight 447 grms., was fed on six consecutive days with the emulsion (obtained by pulping and straining as for *Exp. 1*) obtained from very putrid meat. The first day the method of feeding was by direct introduction into the stomach, but on the other days it was found possible to get the animal to drink the dose (about 20 c.c.) by mixing it with an equal quantity of milk and giving no other food until it was consumed.

Throughout the experiment the animal showed no symptoms and remained active and apparently unaffected. During the period of feeding the gain in weight was only 10 grms., but when killed at the end of 23 days the weight had increased by 52 grms., or 11.6 grms. per 100 grms. of body weight. A control kitten over the same period fed with similar food, but without any putrid meat extract, gained 79 grms., or 15.5 grms. per 100 grms. of body weight. The experimental kitten, when killed, was very thin in appearance and a post-mortem showed a muscular development well below the normal. Microscopically the organs appeared sound. No bacteriological cultivations were made.

While the nutrition of the animal was somewhat interfered with no active symptoms of ill health resulted and nothing in the nature of food poisoning.

Exp. 3. A young kitten, weight 460 grms., was fed on three successive days with putrid meat washings from shop beef allowed to putrefy naturally. Putrid emulsion from about 20 grms. of meat given each day. The animal remained well and increased in weight. Killed 11 days after the last feeding. The animal throughout exhibited no symptoms while post-mortem the kitten was found to be fairly well nourished and showed no lesions.

Exp. 4. A kitten, weight 645 grms., was fed on three consecutive days with an emulsion (obtained as above) from a mixture of two tins of corned beef very badly blown, with putrid very offensive contents. Emulsion from 30 grms. used for each feed.

The meat showed a great variety of bacteria including *B. proteus* but no anaerobes were isolated.

The kitten showed no symptoms of any kind while no naked eye lesions were present when killed nine days after the last feeding. No microscopic lesions observable in sections from the stomach and small intestine.

The animal showed an increase of weight of 72 grms. during the 12 days and this was only slightly less than that of a control kitten.

Exp. 5. This and the next experiment were designed to test the relative toxicity of the bacteria of putrefaction and of their products separately. Fresh meat was allowed to putrefy naturally at 21° C. for several days. An emulsion in warm water was made by mixing and pulping and allowing to stand at 37° C. for an hour.

The very turbid and offensive liquor, after straining through muslin, amounted to about 15 c.c. obtained from 20 grms. of meat. This was centrifugalised for three quarters of an hour and the liquid then filtered through a porcelain filter. A clear sterile fluid was obtained.

A young rabbit injected subcutaneously with 4 c.c. of this fluid remained perfectly well and showed no symptoms.

The centrifugalised deposit was repeatedly mixed with sterile water and recentrifugalised to wash it free from any toxins. Another young rabbit injected subcutaneously with a considerable quantity of this washed deposit mixed with a little water showed no symptoms and remained quite unaffected.

Exp. 6. Raw fresh meat was allowed to putrefy naturally at 21° C. for 48 hours. A very rich bacterial emulsion was then made by scraping off the surface bacteria first, then pulping the meat and keeping it mixed with sterile water, and with the surface bacteria, at 37° C. for two hours. The liquid was then strained through muslin. Three experiments were made:

(a) A rabbit injected subcutaneously with 1 c.c. of the untreated turbid and offensively smelling emulsion remained quiet but not obtrusively ill for the first 24 hours, but was moribund and killed about 48 hours after the injection. A post-mortem examination revealed blood-stained exudation and great redness of the subcutaneous tissue and muscles at the site of the inoculation and *B. welchii* was isolated from this lesion. No abnormalities internally.

(b) The supernatant fluid after centrifugalisation was filtered through a sterile porcelain filter and 3 c.c. of the clear filtrate injected into a young rabbit. No symptoms resulted and the animal remained well.

(c) The centrifugalised deposit after well washing and recentrifuging three times to remove extra-cellular toxins was injected subcutaneously into a guinea-pig (a rabbit was not available). The animal was quiet but not obtrusively ill next day, but was found dead on the following day. Very typical *B. welchii* infection lesions while *B. coli* was also isolated. The small intestine was full of fluid with a strong putrid odour. The other internal organs appeared normal but *B. coli* was isolated from both the liver and the spleen.

Exp. 7—to test the toxicity of the sterile products of putrefaction. Meat, after moistening, was kept at 21° C. in a covered glass jar (*i.e.* under semi-anaerobic conditions) for five days. An emulsion was made in the usual way from the green and very putrefactive meat. The strained liquid was filtered through a sterile porcelain filter, 5 c.c. of it being the filtrate from about 15 grms. of meat, and injected intraperitoneally into a kitten of weight 542 grms. The animal remained perfectly well throughout the experiment but lost 57 grms. during the first seven days following the injection; its weight then slowly increased and 17 days after the injection it had reached the weight recorded at the start of the experiment.

Exp. 8. A young kitten, weight 572 grms., was fed with an emulsion from raw meat allowed to putrefy naturally at 21° C. The emulsions were obtained as in the previous experiments. The first feeding was after two days natural

putrefaction. Six feeds on consecutive days were given, the last therefore being after eight days' putrefaction.

During the six days of feeding the animal gained 5 grms., then alternately lost and gained weight, and when killed 32 days after the first feeding it was 590 grms. In 32 days therefore the gain was only 18 grms. instead of about 160 which would be about the normal growth increase in this period.

The kitten remained perfectly well throughout without any evidence of gastro-intestinal disturbance or other symptoms.

Post-mortem showed the animal to be fairly well nourished with no lesions anywhere, while cultivations (aerobic and anaerobic) from both the liver and spleen were sterile. No putrefactive anaerobes could be cultivated from the small intestine.

A study was also made of the excreta of this kitten during the course of the feeding to try to discover and isolate putrefactive organisms. No putrefactive anaerobes were found but *B. proteus* was isolated. *B. proteus* was abundant in the putrid meat itself but proteolytic anaerobes could not be isolated.

Exp. 9. A feeding experiment with putrid canned meat obtained from a very blown 6 lb. tin with very offensive contents. The bacteriological examination showed *B. proteus* abundantly present and in pure culture, anaerobes and other bacilli being absent. This *B. proteus* strain was pathogenic to a mouse by subcutaneous injection and was recovered from the liver and heart-blood. A kitten was fed on four successive days with the emulsion from about 60 grms. for each feed. No vomiting, diarrhoea or other symptoms of illness occurred while the animal remained lively and active. It, however, suddenly became ill one morning and died seven days after the last feeding.

The kitten weighed 540 grms. at the time of the initial feed and its weight steadily declined to 417 grms., *i.e.* it lost 123 grms. over the 11 days' period between the starting of feeding and death.

Post-mortem the animal was found to be extremely thin, but there were no naked eye lesions in the alimentary tract or indeed in any organ. Aerobic and anaerobic cultures from the liver, spleen and heart-blood showed a few *B. coli* colonies but no *B. proteus*. *B. proteus* could not be isolated from the large or small intestine although it was quite easily isolated from the meat used for the feeding.

Microscopic sections from the small intestine of this animal and also from a number of the other kittens showed no pathological changes.

Exp. 10. Since Experiment 9 suggested some toxicity for *B. proteus*-infected meat, another experiment on similar lines was carried out.

The meat for feeding was obtained from another blown tin of corned meat with extremely offensive contents with abundant slimy growth. No anaerobes could be detected and *B. proteus* was isolated as the only organism present.

The kitten was fed on four successive days with the emulsion obtained from about 60 grms. of the putrid meat. The animal showed no symptoms of

ill health and remained perfectly well. It weighed 767 grms. at the start of the experiment and the weight did not decline at all, but steadily increased and the kitten weighed 960 grms. when killed 26 days after the first feeding.

A post-mortem examination disclosed no lesions anywhere and the animal was well nourished. Aerobic and anaerobic cultivations from the spleen, liver and heart-blood were sterile while *B. proteus* could not be isolated from the contents of the small intestine.

Exp. 11. A more comprehensive feeding experiment in which four kittens were employed. Great care was taken that all four animals received exactly the same quantities of food (mostly milk) in addition to the putrid meat emulsions. All four animals were from the same litter and were of nearly the same weight. The putrid meat used was derived from a very blown tin of corned beef with highly offensive and decomposed contents. The bacteriological examination showed the putrefactive anaerobe *B. sporogenes* in pure culture. Infected intraperitoneally in pure culture into a guinea-pig it was non-pathogenic.

The feeding emulsion, obtained as above, was all prepared at once and kept in flasks and the extract from 60 grms. of meat was used for each feeding experiment. The emulsion dose was given with milk.

Kitten A. Fed on seven consecutive days with the whole emulsion, *i.e.* bacteria and products.

Kitten B. Fed on the same seven days with the sterile filtrate from another portion, filtered through a porcelain filter.

Kitten C. Control, pure food only being given.

Kitten D. The bacteria on the porcelain filter used to filter the emulsion fed to *Kitten B* were scraped off and emulsified in sterile water, several lots of water being used. The mixed emulsions were centrifugalised, washed and recentrifugalised. The bacterial bodies in this way separated from the chemical products of decomposition were then emulsified in a little water and mixed with milk and fed to *Kitten D* on seven consecutive days.

In all cases the milk used for feeding was sweetened condensed milk. The animals were kept in separate cages. *Kitten A* remained seemingly in good health until four days after the last feeding, then appeared less lively and not in good condition. It revived somewhat and appeared better, but seven days after the last feeding showed inflammation of one eye. This became worse and later affected both eyes. The suppurative condition of the eyes increased and the animal was obviously ill. It would take but little food and became moribund and was killed 22 days after the start of the experiment.

On post-mortem the animal was found to be very thin with little or no abdominal fat. There was marked excess of fluid in the peritoneal cavity. The small intestines, stomach, kidneys, liver and other internal organs appeared healthy.

Aerobic and anaerobic cultivations from the peritoneal fluid, heart-blood, liver, and spleen were all negative except that the anaerobic growth upon egg-

meat medium from the liver yielded *B. sporogenes* identical in its characters with the strain found in the putrid meat used for the feeding.

The eye conditions were very marked, both showing severe purulent inflammation with nearly complete corneal opacity in the right eye.

Kitten *B* remained quite well and lively throughout, showing no symptoms at any time. No eye inflammation. When killed was well nourished and showed no abnormalities.

Kitten *C* remained well and lively and showed no symptoms apart from a loss in weight. This loss was not progressive but a big drop of nearly 100 grms. took place between the sixth and tenth days. It then increased fairly regularly in weight, but rapidly lost weight again (94 grms.) during the three days before death: 23 days from the onset of the experiment it was found dead although apparently in good health the day previously.

Post-mortem the animal was found to be thin, but no abnormalities were detected. Animal parasites were looked for, but nothing detected to indicate the cause of death. Cultivations from the internal organs were sterile. There were no eye symptoms or lesions.

Kitten *D* remained well and lively until 14 days after the onset of the feeding when inflammation in one eye was noticed. In a day or two this was marked and purulent and later extended to both eyes. The animal refused to take much food and became ill and was killed in a nearly moribund condition 25 days after the start of the experiment.

Post-mortem both eyes showed a very purulent condition with suppuration of the cornea. The animal was very thin with no fat in the abdominal wall or around the organs. There was no excess of fluid in the peritoneal cavity while the internal organs appeared healthy. Aerobic and anaerobic cultivations from the heart-blood were sterile but *B. coli* was isolated from both the liver and the spleen.

The weight changes in grammes were as follows:

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Weight at onset of the feeding	650	648	623	644
Loss of weight at end of the 7 days of feeding	99	73	13	68
Loss or gain in weight during the 14 days after the cessation of feeding	- 91	+ 117	- 110	- 63
Loss or gain in weight at end of 21 days from start of the feeding	- 190	+ 44	- 123	- 131

Unfortunately the inflammation of the eyes which developed in two of the kittens (*A* and *D*) deprives this experiment of most of its value, but it is included in the series as it has some special points of interest.

Towards the end of the experiment these kittens consumed very little of their milk rations and this and the severe eye conditions are sufficient to account for the loss of weight.

The loss of weight in the control kitten is unaccountable. Part of it, no doubt, was due to the insufficient food given since this animal only received exactly the same amount of food as the others and the quantity of this was

governed by the capability of the kitten which consumed least. The fact that only tinned food was given may have had something to do with it.

The bacterial filtrate evidently had no permanent prejudicial effect upon the kitten so fed (*B*).

From another point of view these eye lesions are of interest since the conditions found almost exactly correspond with the description of xerophthalmia recorded by Osborne and Mendel and by McCollum as resulting in experimental animals supplied only with a diet free from the fat-soluble *A* vitamin substance. As already mentioned the kittens were entirely fed (until after the eye lesions had developed) with condensed milk and corned beef products, neither being fresh foods. I was unaware of this condition until Professor McCollum drew my attention to it in America some months after this experiment was completed, otherwise a more detailed study of the eye conditions would have been made.

That the eye conditions were due to local infection is the more probable explanation and is supported by a further experiment with another kitten carried out some nine months later. In this experiment a young healthy kitten was fed daily with sound shop condensed milk mixed with water, some of the feedings being with sweetened others with unsweetened milk. No other food of any description was given. This diet was continued for four weeks. The weight steadily diminished and after four weeks was reduced from 987 to 932 grms. About 11 days from the onset of the experiment the animal appeared unhappy, lethargic and slow in movement, but recovered again in a few days' time. When the experiment was discontinued the kitten was normal, in good health and at no time developed any eye symptoms.

Unrestricted quantities of the condensed milk were given but the kitten did not like it and frequently would drink but little and this may have been sufficient to account for the loss of weight apart from the absence of suitable vitamins. When transferred to another diet it rapidly put on weight.

Exp. 12. An experiment upon the same lines as *Exp. 11*, but using a different feeding material and with some minor modifications.

The putrid food used was a mixture of four tins of canned marine products as follows:

No. 310. Crayfish. A very blown tin which, when opened, showed a decomposed mass strongly putrefactive. The condition was due to a putrefactive anaerobe (*A. 465*), the only living organism present. The chemical tests showed a very high amino-acid content (*Sørensen* figure) while a positive test for tyrosine was obtained showing that definite putrefactive substances were present.

No. 311. Crayfish. A very blown tin. The contents were strongly putrefactive, although not nearly so broken down as No. 310. No anaerobes were isolated and the bacterial cause of the decomposition was not found, having probably died out. The only organism isolated was an aerobic sporing bacillus of *B. subtilis* type. The tyrosine test was negative but the amino-acid figure was very high.

No. 312. Crab. A very blown tin. Abundant gas escape when opened with a strongly putrefactive odour, the crab meat, however, not being much broken down. Anaerobes

were absent and the only organisms isolated were a sporing aerobe of *B. subtilis* type and a non-sporing bacillus which did not produce gas. The tyrosine test was negative but the amino-acid figure was very high.

No. 313. Salmon. A very blown tin. Abundant gas, with a very offensive odour, escaped when opened. The fish was soft, broken down and indeed pulpy, with gas exudation. Here again the bacterial cause of the condition was not isolated and probably had died out, the only organisms isolated being a diplococcus and a non-sporing non-gas producing aerobe. The tyrosine figure was negative but the amino-acid figure was extremely high.

The mixture obtained from these four samples represented therefore a feeding mixture of high but varying degrees of putrefaction and containing a number of different living bacteria at least one of which was actively associated with the changes. If putrefactive material is prejudicial by feeding to kittens this mixture certainly should have exhibited to the full such harmful properties.

The portions of the four tins not required for the other examinations were emptied out and rubbed up in a mortar with five times their weight of sterile water. The emulsion was kept at 37° C. for one hour. The turbid liquid was squeezed out through fine muslin and the solid remainder again extracted with half its original weight of sterile water for a further hour at 37° C.

The liquid was re-strained through fine muslin and the residue with half its weight of water again extracted. The turbid liquids were all mixed together in a large flask. The weight of fish taken was known so it was possible to arrange that the extract from 20 grms. should be the given dose for each feeding; this amounting to 60 c.c.

The strongly putrefactive, offensive liquid was kept at room temperature and used throughout for the feeding tests.

Four young kittens were used, three being from the same litter, and they were weighed carefully every two days for a week before the experiment started.

Kitten A. Fed on seven occasions out of eight consecutive days with the whole 60 c.c. of emulsion, *i.e.* bacteria + products.

Kitten B. Fed on the same seven days with the sterile filtrate from another 60 c.c. filtered through a porcelain filter

Kitten C. Control. Milk only being given.

Kitten D. The bacteria on the porcelain filter used in connection with *Kitten B* were scraped off into sterile water, several lots of water being used. The bacterial bodies separated from their chemical products were used for the feeding of this animal, and were fed on the same days as for the other kittens.

In this experiment great care was taken to give the kittens exactly the same amount of other food, the food used being 50 c.c. of milk for each animal. This was raised to 100 c.c. as soon as the experimental feeding was finished, while bread or fish was also added, but throughout each animal received rigidly the same quantity of food.

Kittens *A* and *B* found their food distasteful, but they would take it if no other was given. After the first two days it was found simpler to feed these two animals by means of a siphon stomach tube. They took the food very well in this way. There was no difficulty with the bacterial emulsion, *Kitten D* taking it readily when mixed with a little milk.

The kittens were allowed to mix and take exercise together after they had taken their dose as in this way they were livelier and seemed better.

Temperature records were found useless, and the tests relied upon to judge the condition of the animals were their weight, their general condition (liveliness, playfulness, appearance of coat), and finally the post-mortem changes.

The weight changes in grammes were as follows (animals weighed every two days):

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Wt. at onset of the feeding	810	816	728	756
Wt. at end of the 8 days of feeding ..	672	689	650	668
Wt. 3 weeks after end of experiment ...	992	1038	921	967
Loss of wt. at end of the 8 days	138	127	78	88
Gain of wt. in last 3 weeks	320	349	271	291
Total gain in wt. during the whole 29 days	182	222	192	210
Total gain in wt. during the whole 29 days per 100 grms. of body wt.	22.5	27.5	26.5	27.9

Kitten A. Considerable subcutaneous fat, abdominal wall of average thickness, considerable omentum fat and around the kidneys; would not call the animal badly nourished, but was not fat. All internal organs appeared normal. Section of small intestines showed no changes. Cultivations from spleen and liver sterile.

Kitten B. As for *Kitten A*, but rather more fat.

Kitten D. As for *Kitten A*, but rather more fat.

Both *B* and *D* were better nourished than *A*.

Not considered necessary to make a post-mortem examination of the control *Kitten C*.

Exp. 13. A repetition of *Exp. 12*, but with different material. The feeding mixture used was a mixture from four tins of unsound fish products as follows:

No. 334. A very blown tin of sardines. Escape of offensive smelling gas when opened, but the fish itself was very little broken down and with only a slight odour. Bacteriologically it was sterile. Chemically it gave a slight positive tyrosine test while the amino-acid figure was not above the average.

No. 335. A blown tin of salmon. When opened, marked gas escape and the fish had an offensive odour but not marked, while the salmon was not at all broken down. Sporing aerobic bacilli and a streptococcus isolated. No anaerobes isolated. No chemical examination made.

No. 336. A blown tin of salmon. When opened an abundant escape of very offensive gas, while the salmon was broken down and decomposed with a very offensive odour. An anaerobe was present but died out in the attempt to isolate it in pure culture. A streptococcus was the only other organism isolated. No chemical examination made.

Fourth tin. A tin of salmon which was blown and which showed offensive and decomposed contents was inoculated with A. 474, a putrefactive anaerobe isolated from other canned foods. The tin was resealed up and incubated for two days at 32° C. The tin became very blown and when re-opened showed very offensive and still further broken down contents. A. 474 recovered.

The last two tins were selected as examples of very advanced putrefactive changes with the presence of at least one putrefactive anaerobe. The first two tins were used as types of

much less developed putrefactive changes as it is possible that more toxic products might be present in the earlier stages.

The method of making the feeding emulsion was exactly the same as for Exp. 12. In addition, to complete the possible toxicity of the mixture, two broth cultures of two putrefactive aerobes of *B. proteus* type (A. 213 and A. 248) were added to the flask of offensive liquid.

The feeding dose was the same as Exp. 12, while four kittens were used: Kitten *A* received the whole emulsion, Kitten *B* the sterile filtrate, Kitten *D* the emulsified bacteria without the chemical products, Kitten *C* (the control) received no fish extract. The kittens were fed on seven consecutive days with the fish extracts, all the animals receiving in addition exactly the same amount of other food; milk only being given until the end of the experimental feeding, then milk and bread.

The other details were the same as for Exp. 12 except that in every case for Kittens *A* and *B* the method of feeding was by the stomach tube.

The only points noted as regards the condition of the kittens were that on July 13th and 14th (sixth and seventh feedings respectively) Kitten *B*, about a quarter of an hour after feeding, vomited about half or less of the dose and evacuated liquid from the bowel. The animal seemed ill and staggered about but recovered completely after about 20 minutes.

Kitten *A* (seventh feed) showed similar symptoms, but the vomiting was only a little, but no diarrhoea. These disturbances were probably reflex in origin and associated with the method of feeding.

The weight changes (in grammes) were as follows:

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Wt. at the onset of the feeding	710	761	615	683
Wt. at end of the 7 days of feeding	720	723	623	702
Wt. 21 days after end of the experiment ...	1010	935	891	964
Loss or gain of wt. at end of the 7 day. ...	+ 10	- 38	+ 8	+ 19
Gain of wt. in last 3 weeks	290	212	268	262
Total gain in wt. during the whole 28 days	300	174	276	281
Total gain in wt. during the whole 28 days per 100 grms. of body wt.	42.4	22.9	44.9	41.1

Kitten *B* showed some loss, instead of a progressive gain, of weight during the second week after the cessation of feeding.

The post-mortem appearances in Kitten *A* showed a well-nourished animal with a good deal of subcutaneous fat, fat round the kidneys, etc. Internal organs appeared perfectly normal.

It may also be mentioned that a guinea-pig inoculated intra-peritoneally with 1.0 c.c. of the sterile filtrate at the time of the first feeding remained perfectly well.

SUMMARY OF THE ANIMAL EXPERIMENTS.

While not completely concordant, interesting deductions can be drawn. The putrid meat extracts containing both the bacteria and their products, were fatal to two rabbits when the method of introduction was by subcutaneous

injection. A rabbit fed with the same material remained unaffected. On the other hand two rabbits and one kitten injected either subcutaneously or intraperitoneally with the chemical products freed from bacteria showed no ill effects, apart from loss of weight. Of two animals injected with the bacteria washed free from their products one, a rabbit, showed no ill effects, the other, a guinea-pig, died from *B. welchii* infection.

The feeding experiments are mostly in agreement. Exps. 11, 12 and 13 are more conveniently considered together as more complicated in design and arrangement. Apart from these six young kittens were fed on different occasions with meat from different sources but in every case in a definitely putrid condition. For four of them there were no symptoms and no maintained loss of weight although in one (Exp. 11) there was evidence of impairment of nutrition and a restricted gain in weight. In two of the experiments *B. proteus* was very abundant in the fed material.

In one of the remaining two experiments there was a definite loss in weight at one part of the experiment while even after 32 days the animal had only increased 18 grms. There were no symptoms of ill health. In the other there was a marked loss of weight followed by the death of the animal but there were no symptoms of ill health during life. In this case *B. proteus* was abundant in the feeding material.

This suggested toxicity of *B. proteus* was not in accordance with the other two feeding experiments in which this organism was abundant.

Exp. 11 was confused by the eye complications in two of the kittens but Exps. 12 and 13 are very instructive. They were carried out with great care and with every endeavour to eliminate disturbing factors. Both were fed with canned fish in various stages of decomposition. Taking into consideration the variable factors which always affect feeding experiments the results are very concordant and rather striking.

In both sets of experiments most of the eight kittens lost or put on insufficient weight during the actual period of feeding. This was less noticeable in Exp. 13 when it was possible by direct introduction of the food into the stomach to give regularly a larger food ration. The amount of this ration had to be the same for each animal and was liable to be restricted if the kittens took a long time to drink up their putrid emulsions. In Exp. 13 three out of the four showed a gain in weight during this period.

Apart from some loss of appetite all the eight kittens remained well throughout.

In Exp. 12 both kittens *A* and *B* lost considerably more weight than *C* and *D*, but after a further three weeks, *B* had caught up the others. The gain in weight, for 100 grms. body weight, of *B*, *C* and *D* at the end of the experiment is extremely close and shows clearly that given the same amount of food there was no permanent damage to *B*. Kitten *A*, fed with the whole emulsion, still lagged behind in weight. It evidently had not fully recovered the great loss of weight during the actual feeding, but that it was catching up

is shown by the fact that during the three weeks following the feeding its gain per 100 grms. of body weight was 39.3 grms. compared with 42.7, 37.2 and 38.5 for *B*, *C* and *D* respectively. In other words it was putting on weight faster than either the control or the animal fed with washed bacteria.

In Exp. 13, as for Exp. 12, the kitten *D* fed with washed bacteria, behaved exactly like the control and the two experiments show very clearly that the bacteria themselves are without harm and that any prejudicial effect can only be ascribed to the products. In Exp. 13 the three animals *A*, *C* and *D* behaved almost exactly alike, showing almost the same weight increase (8–19 grms. only) during the feeding period, while at the end of the 28 days of observation their gain per 100 grms. of body weight was extremely close. In this experiment the only animal affected was Kitten *B* which not only lost weight during the actual feeding but could not pick up in the subsequent three weeks, its weight increase per 100 grms. for this period being only 27.8 grms. compared with 40.8, 43.6 and 38.4 for *A*, *C* and *D* respectively.

This loss in weight when fed with the filtered products is clearly not a regular phenomenon since it was not observable in Exp. 12 or in Exp. 11, where the kitten feeding for animal *B* was not complicated by any eye lesions.

It must be taken into consideration that the amount of putrid material consumed was enormous, each individual dose being very large, while it was given *daily* over many days. That these heroic doses of such very nauseating substances should have produced so little disturbance of nutrition or loss of weight is striking, and strongly suggests that their toxicity is either entirely absent or of the lowest grade.

In no case (except Exp. 9) was there the slightest evidence of infection or of any ill effects, apart from disturbance of nutrition probably to be accounted for by the nauseating food.

GENERAL CONSIDERATIONS.

The problem of the prejudicial properties of decomposing food can be considered from points of view other than those of direct feeding experiments. If such food possesses the toxicity commonly ascribed to it three contributory lines of study should bring forward evidence to support the contention. It should be possible to isolate the organisms associated with this condition and to show that they are capable when fed of producing prejudicial symptoms. The bio-chemist should be able to separate the products of putrefaction and prove which of them possess disease producing properties. In the third place it should be possible to point to outbreaks of food poisoning or illness in individuals caused by the ingestion of putrid material. These contributory points merit some consideration.

Toxicity of the putrefactive bacteria. We do not know all the bacteria associated with putrefaction and it is doubtful how far it is justifiable to speak of putrefactive bacteria as if they were a separate group. Undoubtedly, however, certain organisms are found commonly associated with the putrefactive

changes in foods and are capable, singly or in combination, of producing the chemical products we associate with putrefaction. The most important of such organisms are *B. proteus* (using the term to include a group of organisms) and the putrefactive anaerobes such as *B. sporogenes* and *B. putrificus*.

The most putrefactive of these anaerobes are non-pathogenic to laboratory animals even when injected and appear to be quite harmless when fed.

The pathogenicity of *B. proteus* is higher as many strains are decidedly pathogenic on injection under the skin, but their pathogenicity by feeding is negligible or absent. With strains isolated from cases of infantile diarrhoea, Metchnikoff and his followers set up in a few instances gastro-enteritis by prolonged feeding, while Herter and Broeck caused diarrhoea with green stools in one monkey out of three fed with a strain of this organism. There is no evidence that I can find showing that strains of this organism isolated from putrefactive material are capable of setting up illness in animals by feeding. In several of the experiments recorded above, *B. proteus* was very abundant in the putrid foods used for feeding.

The study of the chemical products of putrefactive origin may be said to have passed through two phases. The first of these is the one dominated by the ptomaine hypothesis. Earlier investigators, working with a very incomplete knowledge of the decomposition products of the protein molecule, isolated a number of products of the nature of diamines from animal matter which they had allowed to putrefy for long periods and which diamines, when injected into animals, caused marked symptoms usually culminating in death. They were isolated from the late stages of putrefaction and represented late protein degradation products. They were only produced when the food was far too nasty to run any chance of being eaten. The whole of the evidence as to their toxicity rests upon *injection* experiments and there are no facts showing that they have any material toxicity when introduced by the mouth. Ptomaines can certainly be dismissed as having anything to do with the alleged toxicity of tainted meat.

The second phase is one widely accepted but while more scientific in its basis is equally unconvincing. It relies for its evidence upon the fact that certain definite protein degradation products which are of considerable toxicity are manufactured by the activities of putrefactive bacteria and that these might be produced in the early stages of putrefaction. For example it has been shown that the poisonous bases β -imidazolethylamine and tyramine are produced by the action of putrefactive bacteria upon histidine and tyrosine, two amino-acids, early stages in the breaking down of proteins. The evidence, however, as to their poisonous properties is derived from injection and not feeding experiments, while the fact that these poisonous bodies are produced from the cleavage of proteins by bacterial action in the *normal* intestine suggests that the human body possesses a defensive mechanism capable of dealing effectively with them.

There is no evidence, that I am aware of, showing that these poisonous

bodies occur to putrefactive foods and that when fed to animals they exert any poisonous action. In other words investigations upon the chemical products of putrefactive bacilli in meat or other food in a condition in which it would be eaten (the specific food poisoning bacilli are *not* putrefactive bacilli) have failed to show any substance or group of substances capable of originating symptoms of ill health when taken by the mouth¹.

As regards outbreaks of food poisoning¹ a very careful study of the literature leads to the conviction that there are no recorded outbreaks of food poisoning which have been proved to be due to the consumption of food in a putrefying condition unaccompanied by the presence of specific food poisoning bacteria or their specific toxins. Small outbreaks confined to a single case or two or three members of one family are usually not investigated so it cannot be asserted that none of these could be due to the consumption of food in an incipient state of decomposition, but certainly the connection has never been established.

A study of the evidence along these accessory lines of inquiry singularly fails to bring forward any evidence associating the consumption of food in a state of incipient putrefaction with illness in those who consume it.

In support of the argument as to the limited toxicity of putrid food we have the well-known facts that many uncivilised races habitually consume fish, game and other foods in a condition of definite putrefaction, while "civilised" man, in his preference for game which is "high," is following a practice only differing in degree.

SOME ADMINISTRATIVE AND PRACTICAL CONSIDERATIONS.

It may be advanced that a view which minimises or denies the evidence incriminating tainted meat as a cause of illness is a very damaging one administratively and will, or may, lead, if accepted, to a disregard of cleanliness and the consumption of food which is stale or possibly even tainted. It may further be contended that whether the view of the highly dangerous condition of tainted meat is right or wrong the conception has been of great advantage to the administrator and a powerful means whereby improvements have been effected in obtaining a cleaner, fresher and purer food supply.

These are important practical considerations and demand attention.

In the first place the animal experiments recorded do not suggest that food in a putrefying condition is harmless to man and can be neglected. The actual experiments show some loss of weight and disturbances of nutrition in several cases with the very large doses given, and it is not safe to assume without more evidence that the intestine of man is as little sensitive as that of kittens. The report is more particularly intended to draw attention to the absence of positive scientific data incriminating putrefactive food as a cause of definitive illness and to emphasise that this mass action of putrefactive bacteria and their

¹ These two aspects are only touched upon here. They are dealt with in considerable detail in my book, *Food Poisonings and Food Infections*, 1919, pp. 115 *et seq.*

products is a minor, if not a negligible, matter in the production of toxic symptoms in man. Harmfulness from bad food is a matter of specific infection with particular pathogenic bacteria in all or almost all cases.

In the second place if food is found to be decomposed it has evidently been exposed to conditions favouring bacterial infection and such infection may include the special bacteria associated with food infections. Confronted with decomposing food we cannot say without detailed investigation if this is the case or not, so whatever views are held it would be necessary and justifiable to condemn such food as an administrative action.

In the third place the stimulus needed for effective administrative action at the present time is a realisation that food poisoning outbreaks, and attacks of illness generally, from unsound food, are due to infection with specific bacteria in nearly all if not all cases. The present hazy conceptions as to food poisoning and its relationship to tainted food are merely hindrances to effective administrative action. Progress in the prevention of these conditions has been so slow and in the main ineffective because the fundamental importance of specific bacterial infection has neither been realised nor acted upon.

In the end I consider that the administrative control of our food supply will not only not be weakened, but greatly strengthened, by a recognition of the conceptions advanced here.

We have an exact parallel instance in regard to the hypothesis of the close and intimate association between outbreaks and cases of diphtheria with drain air and unsound drains.

This incorrect and exploded hypothesis was undoubtedly the driving cause of an enormous amount of valuable sanitary work being carried out, the broad general effect of which was the improvement of the health of the community. On the other hand, under its blighting influence it caused immeasurable harm. Case after case of diphtheria enquiry ended with the discovery of a broken drain pipe and the true cause was missed since it was never sought for, and the unrecognised infecting case or contact was left to spread the disease broadcast. I have known it advanced that the drain hypothesis, if not true, was at least very beneficial as a lever for good works and that to throw doubt upon it was not in the best interests of public health. As I have mentioned, the parallel is a close one and may be pressed in all its phases. It is the same old quarrel between the general and the specific causation of disease.

AN INVESTIGATION ON INFLUENZA.

BY F. W. TWORT AND D. N. TWORT.

(*From the laboratories of the Brown Institution for the
Medical Research Council.*)

CONTENTS.

	PAGE
I. Introduction	85
II. The bacteriology of Influenza	86
III. Conditions influencing the toxicity and pathogenicity of the influenza bacillus	86
IV. Filter-passing substances which break down and dissolve bacteria	87
V. Special forms of bacteria in pure cultures, and the possibility of the existence of sexual units	88
VI. Toxicity of the special forms	89
VII. Experiments on the cultivation of non-pathogenic ultra-microscopic viruses under the influence of various gases	91
VIII. The possible influence of light rays and electrical changes	95
IX. The cultivation of the influenza bacillus in symbiosis with amoebae on blood agar, etc.	95
X. The cultivation of the influenza bacillus in symbiosis with certain bacteria from grass and soil on media containing no blood	97
XI. The cultivation of the influenza bacillus in an atmosphere of pure oxygen and pure carbon dioxide under diminished pressure	97
Summary	97

I. INTRODUCTION.

DURING the past year we have continued our investigation on influenza. The work may be divided into two sections: that which was carried out on cases of influenza, and with micro-organisms isolated therefrom, and that which was made with the object of throwing light on the subject by more indirect experiments.

Throughout the investigation attempts have been made to open up some entirely new lines of research, as were suggested by fundamental biological laws and conceptions, or those which might exist in theory, rather than to extend in more minute detail along lines that have now been followed without much change for many years, and are usually considered more orthodox. This method of investigation has been carried out because while orthodox methods are easiest to follow and may perhaps lead to small improvements in our technique, and may be followed by some increase in our knowledge, nevertheless it appears to the writers that, speaking in general terms, the usual lines

of investigation have now carried our knowledge nearly as far as such methods are likely to lead. On the other hand, if any fundamental advance is to be made, some entirely new lines of investigation must be followed; and although research of this nature usually gives negative results, it is, however, more likely to lead to important advances in our knowledge.

II. THE BACTERIOLOGY OF INFLUENZA.

The preliminary work consisted for the most part in isolating every possible micro-organism from a few selected and typical cases of influenza. It will not, however, be necessary to go into these in detail, it is sufficient to note that the various micro-organisms isolated were similar to those usually met with by other workers. The influenza bacillus often associated with pneumococci, streptococci, and micrococci were most frequently encountered, and no evidence was obtained that the rarer types isolated, such as yeasts, coliform and diphtheroid bacilli, etc., had any direct relation to the disease.

All experiments carried out to test for the presence of an ultra-microscopic virus gave negative results. Mice, rabbits, and guinea-pigs failed to develop any condition resembling influenza when they were inoculated with filtrates obtained from influenza material, various methods of inoculation being employed. The pneumococci and streptococci isolated showed the usual pathogenic action on mice and rabbits. Inoculations carried out with different strains of the influenza bacillus on mice, rabbits and guinea-pigs showed no marked pathogenic action for these animals. With small doses there was merely some rise of temperature accompanied by a certain amount of malaise which, however, quickly passed off. With large doses mice and, to a less extent, rabbits showed more marked illness, and in the case of rabbits the temperature sometimes became subnormal. The animals, however, were usually well again by the second or third day. No monkeys were tested.

Our standard medium throughout this work for the isolation and cultivation of the influenza bacillus was prepared as follows:

Ordinary peptone-beef-broth was sterilised in a flask, and to this when cool was added 5 per cent. of rabbit's blood, withdrawn from the vein of the ear with a sterile syringe. The whole was then incubated at 37° C. for one hour, repeatedly shaking to prevent the formation of much clot. About 20 drops of this fluid were added to ordinary agar tubes which had been melted and cooled to about 50° C. The tubes were then sloped ready for use. This proved a very satisfactory medium, and was free from the possibility of containing contaminating micro-organisms.

III. CONDITIONS INFLUENCING THE TOXICITY AND PATHOGENICITY OF THE INFLUENZA BACILLUS.

A considerable number of experiments were carried out to investigate the conditions under which cultures of the influenza bacillus might produce more toxic substances or become more pathogenic. In the first place fresh tissues and organs of normal mice and rabbits were removed immediately after the

animals had been killed, and were placed into test tubes containing a little sterile normal saline. Cultures of the influenza bacillus on such media were inoculated into mice and rabbits after one or more days' incubation of the cultures at 37° C. The results were not very definite, but cultures of the bacillus, grown in fresh kidney and liver media, appeared to be somewhat more toxic for the animals than cultures made in other media. Further experiments were then carried out to test the effect on toxin production, etc. when the media, instead of containing normal liver or kidney, contained one or other of these organs which had been taken from a mouse or rabbit which had previously been inoculated with a large dose of the influenza bacillus. Cultures obtained on such media appeared to show a distinctly more pathogenic action for the animals than did the previous cultures. This was what was expected on theoretical grounds, but experiments of this nature are complicated and possess so many obvious pitfalls that one would not be justified in drawing any definite conclusions until all possible errors and flaws have been investigated. Again, the possibility of combinations in such experiments is obviously very great, and if further investigation should support the general results suggesting increased toxicity and pathogenicity, it would still be necessary to work out the best conditions favouring these ends. If really toxic substances could be produced by some such means it might be of value in the preparation of vaccines and possibly also of an immunising serum. In the experiments described the best results were obtained by using the organs of the same species of animal as that to be afterwards inoculated. Two of the possibilities that were being tested were, firstly, does a preliminary dose prepare the organs so that they may break down and set free the bacillary toxins? or secondly, does the toxic effect come from the tissues that have been changed by the bacilli?

IV. FILTER-PASSING SUBSTANCES WHICH BREAK DOWN AND DISSOLVE BACTERIA.

Since 1913 one of us (F.W.T.) has been investigating the biology of certain bacilli, notably members of the dysentery-typhoid-coli group, and the results are in course of publication¹. This investigation may have some bearing on certain aspects of influenza, so the main results and theoretical considerations may be referred to here. Before dealing with this paper, however, it may be mentioned that one of us (F.W.T.) has shown² that "pure" cultures of bacteria may be associated with a filter-passing material which may entirely break down into granules the bacteria of the culture. This was most definitely demonstrated in cultures of micrococci which were isolated from vaccinia. Such cultures when plated out grew colonies, some of which could not be sub-cultured, but if kept they became glassy and transparent. The material of these colonies, when diluted about a million times and passed through a fine

¹ Researches on Dysentery, *Brit. Journ. of Exper. Pathol.* October, 1920.

² An investigation on the nature of Ultramicroscopic Viruses, *Lancet*, December 4th, 1915.

porcelain filter, was capable of infecting a fresh growth of the micrococcus, and this condition or disease can be transmitted to fresh "pure" cultures of the micro-organism for an indefinite number of generations, although the transparent material will not grow by itself on any known medium. The evidence obtained from the experiments supported the hypothesis that this material was not an ultra-microscopic virus growing on the living micro-organisms, but was a ferment secreted by the micro-organism for some purpose not altogether clear. A similar dissolving material was also found associated with the dysentery-typhoid-coli group of bacilli. Dr F. d'Herelle, at the Pasteur Institute, has confirmed these results in cultures of dysentery bacilli, although he believed that the dissolving material was an ultra-microscopic virus.

V. SPECIAL FORMS OF BACTERIA IN PURE CULTURES, AND THE POSSIBILITY OF THE EXISTENCE OF SEXUAL UNITS.

To return to the investigation on dysentery, it is a well-known fact that various species of the dysentery-typhoid-coli group, when apparently in pure growth, produce forms that are considerably larger than the typical short bacilli, and variations in the size and shape of individual bacilli in a pure culture have often been described. Recently Major Hort and others have carried out a number of experiments with the object of throwing light on the significance of these different forms.

In certain experiments dealing with the dissolving material, one of us (F.W.T.) observed these long forms in greater number than usual and it was thought that they might in some way be associated with the dissolving material which had been found associated with this group. On the other hand, there was the obvious possibility that they might be distinct but symbiotic bacilli, or again they might represent a stage in the life history of the bacillus. It was also thought possible that they might be special forms with special work to do, like bees in a swarm, and might prove to be of importance in connection with the pathogenicity of the cultures and the production of immunity in the host, or they might represent nothing more than a mutation.

These large forms, after some difficulty, were isolated in apparently pure growth on a special litmus-maltose-agar medium. They were obtained from cultures of dysentery, typhoid and certain coliform bacilli. Further experiments with the Shiga type of dysentery bacillus showed that three fairly distinct types of the large or special forms could be isolated from the original "pure" cultures. Type A consists of long moderately thick threads which are sometimes twisted like a spirochaete. Type B shows long thick threads which are often markedly swollen either in the middle or at one end. The swollen portions may be split open setting free numerous granules, and a considerable number of these granules can be found free in most film preparations. Globular forms are also present, and these vary in size from that of a large coccus to that of a yeast cell. Type C consists of large bacilli which, however, are some-

what shorter than those of type B. The same swollen forms and free granules are present, and branching units are often observed, but in older cultures nearly all the bacilli are replaced by globular forms, often much larger than a coccus, and in these round forms the protoplasm is frequently collected as a semilunar mass round the circumference of the cell.

It may be noted that the large or special forms can always be isolated from normal cultures, and they appear to retain their characters after isolation and do not give rise to normal bacilli. Fermentation tests and agglutination reactions show but little variation from those of the original normal culture. It is clear from the experimental results that the special forms are not, as was at one time thought possible, distinct symbiotic bacilli, but are produced by the normal bacilli of the culture from which they were isolated. On the other hand the special forms do not appear to represent a degeneration, since they are best obtained by using good media and by keeping the cultures under good conditions.

It is of course possible that bacteria, like *Spirogyra* and other low forms of life, may multiply sexually as well as asexually, and the special forms already described may represent the sexual units. It is possible that a sexual form of multiplication is essential to the maintenance of a vigorous life after a certain number of asexual divisions. However, no experimental support to this theory could be obtained. Mixtures of the special forms, after repeated sub-culture in broth, failed to give colonies of normal bacilli, and it appears probable that if sexual forms exist they must be present in the normal culture, since it is from this that the special forms are produced. On the other hand the fact that the special forms cannot be induced to give rise to colonies of normal bacilli, is a strong argument against their being stages in a true life cycle. They may, of course, represent nothing more than mutations, but, if so, it is rather strange that although present in young normal cultures, yet they never appear to survive under ordinary conditions when a normal culture is grown on the usual laboratory media. It may be that a "dissolving material" plays a part in the disappearance of the special forms soon after they are produced.

VI. TOXICITY OF THE SPECIAL FORMS.

Another possible explanation is that the large forms are specialised units which are produced by the normal bacilli for some special purpose. They may assist in producing assimilable food or protective substances for the normal bacilli whose chief function is to reproduce. If such is the case, it might explain why the special forms do not survive in cultures made on laboratory media unless they are isolated from the standard small type. It might also explain certain points regarding successful infection, incubation period, the termination of infection, and the production of toxins, antitoxins, etc. In this connection it is interesting to note that in specimens from the human body of such material as faeces and urine, long forms of bacilli are often present in considerable number, and it does not seem at all impossible that these forms

may play an important rôle in producing the toxic effects in such conditions as infantile diarrhoea and vomiting and cystitis, etc.

Experiments were carried out to test the toxicity of the special forms for animals. The results appeared to show a greater toxicity of these forms than the normal cultures, but they were not sufficiently marked to draw any definite conclusions. It is, however, worthy of note that the special forms appear to lose their toxicity rapidly when they are isolated from the small forms, and it may be that their power of producing toxic substances is fully developed only in those units which are produced directly from the small bacilli. The fact that in a normal culture the special forms are always produced in this manner, and then die out, gives support to this suggestion. If effective toxin-producing units must be produced directly from the small bacilli, it is possible that the use of the dissolving material is to destroy them after they are no longer of any use to the bacterial community, or the dissolving up may be a means of setting free the toxic substances. In the case of the micrococcus which was isolated from vaccinia, the breaking down of the organisms may extend to the whole culture, and in a few hours there may be no units left, but the conditions of cultivation outside the body are very artificial, and it seems improbable that in the infected body this process would extend further than was beneficial to the life of the bacterial community.

If then, as seems possible, the function of some of the special forms is to produce toxins or other substances for the protection of the bacterium, then any conditions which might favour the growth of these forms would assist the normal bacilli to survive and multiply when they gained entrance to the human body. In this connection we carried out a series of experiments to test for the production of special forms in media containing a high percentage of sodium chloride, the idea being that the climatic conditions that favour an outbreak of dysentery would also produce a higher concentration of salt in their environment. In these experiments special forms were produced as in the previous ones, but in addition a few extremely large branching threads were observed. They looked more like the mycelium of a mould than anything else and contained a granular faintly staining protoplasm. The experiments were repeated several times with the same results, but attempts to isolate these forms failed, and their significance could not therefore be determined. It may be noted, however, that great care was taken to eliminate the possibility of contaminations.

In view of the results obtained with dysentery and allied micro-organisms it was considered advisable to carry out somewhat similar experiments with the influenza bacillus, where units of considerable size are occasionally to be seen in cultures. Most media, as we know, are unsuitable for the growth of this micro-organism, so that possible changes in its constitution are strictly limited. On several occasions, however, we were able to isolate colonies that showed a considerable number of large forms, some of which were distinctly swollen in places. These bacilli, after sub-culturing several times on to our

standard influenza medium reverted back to the small forms. This, of course, may have been due to their never having been properly isolated from the small forms, but, be that as it may, it prevented further experiments with the cultures.

We then pursued our investigation in another direction. It would seem that infections of the kidney and possibly also of the liver play an important part in the toxic manifestations seen in an infected animal, and, if it be the function of some special form to produce toxic substances, we thought that these forms might be found in the kidney or liver. Cultures were accordingly made from these organs in a few post-mortem cases of influenza, and in one instance, a colony of an extremely large influenza bacillus was obtained. This strain, however, after being sub-cultured a number of times, reverted back to the small form as in the previous experiments, and this took place before we had an opportunity of testing its toxic action on animals. So far then we have been unable to obtain special large forms of the influenza bacillus showing the same degree of stability as in the case of the dysentery bacilli, but the experiments are being continued.

VII. EXPERIMENTS ON THE CULTIVATION OF NON-PATHOGENIC ULTRA-MICROSCOPIC VIRUSES UNDER THE INFLUENCE OF VARIOUS GASES.

The section of this investigation carried out by more indirect methods was chiefly confined to work on the general character and behaviour of ultra-microscopic viruses. It is obvious that if any light can be thrown on this group in general it will be of assistance in deciding more definitely the ultra-microscopic theory of influenza outbreaks, and in dealing with this group there are many reasons for believing that it can be best attacked by investigating certain other conditions. In the first place there is no definite evidence that such a virus exists in the case of influenza, and even if it does exist it would probably be more or less specific for man, and would therefore be difficult to demonstrate by animal inoculations. Certain diseases of animals, such possibly as vaccinia, should therefore give a greater prospect of success.

At the same time it is well known in the case of ordinary bacteria that every species that has a pathogenic variety has one or more non-pathogenic or wild varieties, and it may reasonably be supposed that the same general rule holds good with the ultra-microscopic group, various species of which are known to infect both animals and plants. Varieties of bacteria which are accustomed to grow outside the body and lead a non-pathogenic existence are usually much more easily cultivated than the strictly pathogenic varieties, and again it is not unreasonable to assume that this would also be the case with the ultra-microscopic group. The non-pathogenic series, which probably exists in such situations as water, soil, faeces, etc., would therefore appear to offer the greatest scope for successful cultivation, although it must be admitted that most if not all of such varieties would probably be very difficult to demonstrate by animal inoculations, unless any means could be adopted so to increase

their virulence by passage or otherwise that pathological lesions could be produced in animals.

Although it has often been claimed that certain pathogenic ultra-microscopic viruses undergo some multiplication on various media, more particularly on that of Noguchi, yet no definite growths, visible to the naked eye, have ever with certainty been obtained with any member of this group. This fact, in view of the extensive distribution in animals and plants of these viruses, suggests most strongly that the failure to obtain growths is not due to any delicacy in their requirements of food supply, but on the other hand is probably a result of some fundamental difference in their physiology. On the other hand, it must be remembered that if the living organic world has been slowly built up in accordance with the theories of evolution, then a bacterium and an amoeba must be highly developed organisms in comparison with much more primitive forms which once existed and probably still exist in nature. It may be that an ultra-microscopic virus belongs somewhere in this vast field of life which is even less organised than the bacterium or amoeba.

The work we have carried out on this group has been done under the influence of the above theoretical considerations. The first experiments, dealing with the possibility of an "essential substance" being the missing requirement for successful cultivation as was shown by one of us to be the case with John's bacillus (*Proc. Roy. Soc.* 1910), were carried out for the Local Government Board and were published in the *Lancet*, Dec. 4th, 1915. As the experiments were all negative they need not be referred to again here, and for the time being it was not considered profitable in connection with the present research to pursue this line much further, but rather to probe in several new directions. In the few experiments we carried out, no results of any importance were obtained.

Bacteria may be divided into aerobes and anaerobes, but many species are known to grow either with or without oxygen, while others may be best cultivated under a partial anaerobic condition. Pure oxygen and carbon dioxide are usually very detrimental to growth. Most bacteria, however, obtain their energy through the oxidation of some carbon compound.

In the case of some of the nitrifying bacteria, carbon dioxide is utilised as a source of carbon, and the energy required to split up the molecule of this gas is obtained by the oxidation of nitrogen.

We know also that other gases are essential to the cultivation of some species. This is notably so in the case of the sulphur bacteria which inhabit certain strata of water from which they can obtain oxygen from above and sulphuretted hydrogen from below. In this instance a certain tension of oxygen is required, and according to the vigour with which the sulphuretted hydrogen is given off from the organic material or sulphides in the mud, so the plane of bacterial growth rises or falls in the water. The iron bacteria are somewhat similar in their physiology, only bacteria of this group obtain their energy by the oxidation of soluble compounds of iron.

It was in view of these and other instances where special gases, etc. are required by bacteria that we started an investigation along similar lines with the ultra-microscopic viruses, bearing in mind that the living tissues of animals and plants at times give suitable conditions for their growth. A considerable number of experiments were carried out in atmospheres consisting of various mixtures of air, carbon dioxide, oxygen, nitrogen, ammonia, etc., but the results up to the present time have proved negative.

Some additional experiments were made with sulphuretted hydrogen and with media containing sulphur compounds, and as we obtained with these what at first appeared to be growths of more than one ultra-microscopic virus, it may be advisable to describe these experiments in some detail.

In the first experiments our standard blood agar medium was used as a basis, the only modification being that 0.05 per cent. of sodium sulphide was added before the addition of the fluid containing blood. On tubes of this medium inoculations were made from the filtrates of emulsions of garden soil obtained by passing the emulsion through an English Berkefeld filter. The tubes were incubated at 37° C. in an atmosphere of air expired from the human lungs. On the first cultures a few minute "colonies" appeared in two days, and these when sub-cultured on to fresh tubes of the same medium grew as a number of very minute colonies along the needle track. Uninoculated tubes which were streaked down with a sterile platinum loop showed no evidence of colonies. Further experiments showed that an emulsion of these supposed colonies when passed through a porcelain filter grew similar colonies when inoculated on to the same medium, although very few were present in the primary cultures. The power of producing colonies, however, appeared to be destroyed when the emulsion was heated to 80° C., but not so when heated to 70° C. The "colonies" grew best at 40° C., and the growth at room temperature was very slow, while at 60° C. no evidence of growth was obtained. Films were made from the colonies by every means considered likely to give stained preparations, carbol fuchsin, giemsa, and Zetnoff's flagella stain being amongst those tried. In every case, however, we were able to detect nothing but extremely minute granules. Hanging drop preparations also gave negative results. In the meantime somewhat similar "colonies" were grown from filtrates obtained from other materials, such as vaccinia, dung, and rotten apples. In view of the importance of deciding definitely whether any formed bodies could be demonstrated by the ultra-microscope, dark ground illumination, or by some photographic process, a number of "cultures" were handed to Mr Barnard who was working on this branch for the Medical Research Council.

So far the experiments had given satisfactory results, and it was hoped that we were dealing with a group of filter-passing viruses which were thermophilic, and non-pathogenic, and which grew in an atmosphere containing an excess of carbon dioxide. Further investigation, however, soon showed that an atmosphere of expired air was not necessary for the formation of "colonies," but that this took place equally well under anaerobic and aerobic conditions.

Later we found that it was not necessary to add fresh blood to the medium, or in fact blood in any form. On the other hand, when the sodium sulphide was replaced by sodium sulphite in the medium no growth took place, and this was also the case when the percentage of sodium sulphide was increased to 0.5 per cent. In the latter case, however, it was thought possible that the alkalinity of the salt might be a detrimental factor in the experiment. This proved to be the case, for when the medium was neutralised after adding the sodium sulphide, it was found that 0.5 per cent. gave rather better results than 0.05 per cent.

A number of animals were inoculated with the supposed growth, but the results were entirely negative.

During these experiments we constantly put up controls which consisted either of uninoculated tubes or those which had been streaked down with a sterile platinum loop, and in all the first experiments the controls failed to give any evidence of growth, no colonies appearing on any of the tubes. In subsequent experiments, however, we found that when the controls that had been streaked down with the sterile platinum loop were left in the incubator for several days and then sub-cultured on to fresh tubes, a few minute colonies appeared along the needle track. These when again sub-cultured produced an abundance of minute colonies. Similar results with the control tubes were obtained on several occasions, and while the possibility of a contaminating ultra-microscopic virus could not be definitely excluded, nevertheless the evidence was against this being the explanation. The air of course may be loaded with non-pathogenic viruses of this nature, and if such were the case we do not know how far they could be excluded from a culture tube when the ordinary bacteriological technique is employed. On the other hand a more probable explanation of the results appeared to us to be that the colonies were nothing more than minute grains of sulphur which for some reason had started to be deposited on the surface of the medium. In view of the nature of the sulphur compound that was being used this possibility had been kept in mind throughout the experiments, but the fact that the medium was clear, that tubes streaked with a sterile platinum loop in the first experiments showed no evidence of colonies, and that in the tubes inoculated in sub-culture from the various filtrates the colonies remained confined to the needle track, in conjunction with the other experiments already described led us at first to think that even if sulphur formed part of the colonies, nevertheless this might have been deposited by the growth of some virus, in a manner similar to that which occurs with the sulphur bacteria.

We know of course in the case of solutions of various salts that some minute crystal or particle of dust is required to start the process of crystallisation, and a phenomenon of this nature is probably the correct explanation of the results described in these experiments. In order to test this point we inoculated some sterile tubes of our medium with some minute particles of sterile sulphur, and in sub-culture from these tubes we obtained minute colonies similar to

those obtained from filtrates of soil, etc., but in the primary cultures there were only a very few, and it was only in the sub-cultures that they were easily detected.

We found also that it was not necessary to add sodium sulphide to the medium, but that the "colonies" would appear on ordinary agar if sub-cultures were made on this medium and the tubes placed under a bell-jar which also covered a dish containing a solution of sodium sulphide to which had been added a small quantity of dilute hydrochloric acid.

VIII. THE POSSIBLE INFLUENCE OF LIGHT RAYS AND ELECTRICAL CHANGES.

Light is known to have an important influence on the growth of bacteria, and while most grow best in the dark, there are certain species such as *Bacterium photometricum* that are favourably influenced by certain light rays. This is sometimes also true with fructification, and may be demonstrated with the species *Philobolus microsporus*. Unless exposed to the light for a few hours the mycelium of this fungus is said to remain barren. The blue-violet and the yellow-red rays of the spectrum usually act quite differently on different species of bacteria. In view of the known action of light and other rays on the physiology of bacteria we have started some experiments along similar lines with the ultra-microscopic group, but so far without obtaining any positive results.

A few experiments have been carried out on the influence of certain electrical changes, but these also have given entirely negative results. Attempts were also made to cultivate an ultra-microscopic virus on certain media under the influence of the growth of bacteria or amoebae, but no evidence of successful cultivation was obtained.

IX. THE CULTIVATION OF THE INFLUENZA BACILLUS IN SYMBIOSIS WITH AMOEBAE ON BLOOD AGAR, ETC.

In other experiments attempts were made to cultivate the influenza bacillus with certain amoebae. It is well known that some non-pathogenic amoebae may be cultivated on an agar medium that is poor in nutriment, and the medium described by Musgrave and Clegg is often used for this purpose. The amoebae grow only with bacteria and frequently with those which belong to the coliform group. If such a culture of an amoeba is transplanted to ordinary peptone-salt-broth agar, the amoebae die out, and the explanation sometimes given for this is that an amoeba will not tolerate a highly nutritious medium. On the other hand the pathogenic amoebae, classified in the sub-group of *Entamoebae*, have never been grown for certain with bacteria, either on highly nutritious or on poorly nutritious media.

After some consideration we were led to favour the view that the failure to grow amoebae on highly nutritious media was probably due entirely to toxic

substances produced by excessive bacterial growth, and, further, that the reason that a coliform type of bacillus is frequently associated with the amoebae might be due partly to the fact that they outgrow the more delicate types, and partly because some of the pathogenic bacteria and other delicate types fail to grow in a medium that is poor in nutriment. It must also be remembered that bacteria grow in advance of amoebae in cultures, so that the cultivation of amoebae with delicate pathogenic bacteria on rich media could scarcely be expected unless special precautions were taken.

Experiments were carried out along the lines indicated above. We had in the laboratory an interesting amoeba that one of us (F.W.T.) had isolated from the faeces of a patient in Salonika. It was isolated on a special medium, but unfortunately we cannot give the exact composition as the Salonika notes have been mislaid. However, this is possibly of no importance as the amoeba has now been growing for several years on a plain water agar medium containing 1 per cent. of ordinary broth.

The elimination of the vigorous growing symbiotic bacteria at first presented some difficulty owing to the presence of a very motile bacillus. The following method, however, gave successful results. The associated bacteria were plated out on ordinary agar and two types isolated, one of which was very motile and the other less so. The less motile variety was then cultivated on Musgrave's medium, and a two days old culture was inoculated on the lower part of the streak with the amoeba-bacterial mixture, and the tube again incubated at 37° C. The upper part of the tube, having already a growth of bacterium upon it, proved less suitable for the more motile bacillus to grow up, and more suitable for the spreading growth of the amoeba, with the result that the amoebae rapidly grew away from the mixed bacterial growth below to the pure bacillary growth above, whence it was easily obtained in sub-culture with the less motile bacillus only. By using the same method it was found possible to eliminate the single type of bacillus from the culture, and substitute the influenza bacillus, providing a good blood agar medium was used, such as that described at the beginning of this paper. A two days old culture of the influenza bacillus on this medium was touched on the lower end of the streak with the amoeba growing with the single bacillus. The amoeba rapidly grew up the streak of influenza, and sub-cultures taken from the upper part showed amoebae and influenza bacilli only. From this culture it was found easy to eliminate the influenza bacillus and substitute such bacteria as typhoid, dysentery and spirilla if the same method was adopted, and it was particularly easy if ordinary agar or Dorset's egg medium was used, as on these media the influenza bacillus will not grow. The exact nature of the amoeba has not yet been settled, but it does not show the characters of *Entamoeba histolytica* or *E. coli*. It is interesting to note that the influenza bacillus appears to benefit by the presence of the amoeba, and it remains alive for a longer period than when grown without the amoebae. This then may be considered a case of symbiosis. Experiments on the production of toxin and various

immunity tests will now be carried out with such mixed cultures, and the research in other ways continued in the manner indicated in this paper.

X. THE CULTIVATION OF THE INFLUENZA BACILLUS IN SYMBIOSIS WITH CERTAIN BACTERIA FROM GRASS AND SOIL ON MEDIA CONTAINING NO BLOOD.

Many writers have pointed out that haemoglobin is essential for the cultivation of *B. influenzae*, but in some quite recent experiments I have found that it will grow on ordinary agar and on egg media without blood if it is grown with certain spirilla obtained from grass and certain extremely minute bacteria obtained from soil which pass a Berkefeld filter with some difficulty. The growth of these micro-organisms is very delicate, except when cultivated with the influenza bacillus, the combined growth being vigorous, dense, heaped up, and becoming brownish in colour. In such mixed cultures on Dorset's egg medium the influenza bacillus will live for five or six months, after which period it can easily be isolated on blood agar. The influenza bacillus has been isolated from mixtures with the assistance of these symbiotic bacteria. These results have been confirmed by repeated experiments with different strains of influenza bacillus. The results show how it may be possible for the influenza bacillus to survive outside the human body, and indicate other possible lines of research. Fuller details will be published later (F.W.T.).

XI. THE CULTIVATION OF THE INFLUENZA BACILLUS IN AN ATMOSPHERE OF PURE OXYGEN AND PURE CARBON DIOXIDE UNDER DIMINISHED PRESSURE.

Further recent experiments on the growth of the influenza bacillus under the influence of certain gases have shown that this micro-organism will grow on blood agar in an atmosphere of pure oxygen or pure carbon dioxide if the pressure is reduced so that there is an external atmospheric pressure over the internal of 450 mm. of mercury. Many other bacteria will also grow in such gases under reduced pressure.

SUMMARY.

(1) Our experiments support the view that influenza is caused by *B. influenzae*, and that pneumococci and certain streptococci are the most important agents of secondary infections. No new type of bacterium was discovered, and no evidence was obtained of the presence of an ultra-microscopic virus.

(2) *B. influenzae* appears more toxic for rabbits and mice when grown on fresh liver or kidney media than when grown on blood agar or in blood broth, and this is especially so when the kidney or liver is obtained from an animal of the same species that has previously been inoculated with a culture of the influenza bacillus.

(3) Three fairly distinct special forms have been isolated from cultures of *B. dysenteriae*. They are probably not sexual units or stages in a true life cycle. Special large forms have also been obtained from cultures of *B. influenzae*, but these reverted back to the normal small type after several sub-cultures.

(4) A filter-passing material has been found associated with certain micrococci from vaccinia and in pure cultures of members of the dysentery-typhoid-coli group of bacteria. This material breaks down and dissolves the bacteria of the cultures, and the "infection" can be carried to fresh normal cultures. The evidence is against its being a living ultra-microscopic virus infecting the bacteria, but it may have some connection with the special forms. No definite evidence of a similar dissolving material has been found associated with cultures of the influenza bacillus.

(5) There is some indication that one or more of the forms isolated from pure cultures of dysentery bacilli may be special toxin-producing units, also that this function is at its maximum when the bacilli are first produced by the normal small forms or by sexual units of a normal culture. This may possibly be the case also with special forms that have been found in cultures of the influenza bacillus.

(6) It is believed that non-pathogenic wild varieties of ultra-microscopic viruses must exist in nature, and that they should present less difficulty in cultivation than the pathogenic varieties. Cultivations were made from filtrates of soil, faeces and water on various special media, and the tubes incubated in various gases. The chief gases tested either alone or mixed were oxygen, nitrogen, carbon dioxide and sulphuretted hydrogen. All the results were negative, but some interesting deposit colonies were obtained on media containing a small quantity of sodium sulphide.

(7) The influenza bacillus grows in symbiosis with amoebae on blood agar, and in such cultures the bacillus lives considerably longer.

(8) The influenza bacillus grows in symbiosis with a small spirillum that was isolated from a grass emulsion, and in symbiosis with an extremely minute and delicate bacterium isolated from garden soil; after passing the emulsions through a Berkefeld filter. With these delicate bacteria the influenza bacillus will grow on media containing no blood, and if an egg medium is used the mixed growth is dense, heaped up and brownish in colour. In such cultures the influenza bacillus may live for months.

(9) The influenza and other bacilli will grow in an atmosphere of pure oxygen or pure carbon dioxide under diminished pressure.

THE TOXIGENIC FEATURES OF STRAINS OF THE DIPHTHERIA BACILLUS ISOLATED FROM HORSES AND FROM A MULE.

By G. F. PETRIE, M.D.

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IN December, 1920, Capt. F. C. Minett, R.A.V.C., of the Royal Army Veterinary School, Aldershot, published an account of diphtheria bacilli isolated by him from eleven horses and one mule. He states that during the last year of the War and for some months after the Armistice the laboratory at the Army Veterinary School received for examination numerous specimens of pus derived chiefly from suspected cases of ulcerative lymphangitis; a condition in horses in which swelling of the lower parts of a limb or limbs is accompanied by abscess formation followed by ulceration. From this material he collected and investigated a number of diphtheroid strains including the bacillus of Preisz-Nocard and in the course of his inquiry discovered twelve strains of the diphtheria bacillus.

Nine of the strains were cultivated from lesions of the type named; the source of the remaining three is given below.

(1) A swab from a trephine opening in the cheek of a horse. A trephining operation had been practised prior to February, 1920, to relieve a nasal discharge. Although the discharge lessened and the animal remained in excellent condition the operation wound proved obstinate in healing and was still open on 17 October, 1920, when *B. diphtheriae* and streptococci were isolated from it. (Culture D 34 referred to below in the text and in the tables.)

(2) Pus from acneiform skin lesions. These consisted of a dozen superficial suppurating areas covered by scabs and mostly confined to the region of the withers; each was about the size of a threepenny bit.

(3) Pus from a contused fetlock. The lesion was not regarded as a lymphangitis.

Capt. Minett gives a detailed description of the characters of the twelve strains and states that five of them proved to be toxic; the remainder yielded no demonstrable toxin. He kindly sent six of the cultures to this laboratory; our thanks are due to him for the opportunity of examining them.

Particulars of the source and date of isolation of the six cultures forming the basis of the present communication are given in Table I; the symbols chosen by Capt. Minett to designate the cultures have been retained.

Microscopically the cultures are in no way distinguishable from the diphtheria bacillus. When grown for toxin production in Erlenmeyer flasks all give a typical surface film and in the case of "L" culture—the one mostly worked with—the "curtains" characteristic of a heavy, coherent, rapidly growing pellicle of the *B. diphtheriae* are in some batches exceptionally well developed.

Daily estimations of the hydrogen-ion concentration during the period of growth of a number of batches inoculated with cultures "L" and "D 34" were made; the resulting curves resembled those of standard toxin-producing strains of the *B. diphtheriae*.

All the strains formed acid from glucose and maltose but not from mannite and saccharose.

The data concerning toxin production are arranged in Tables II to VIII; and the results obtained on the first attempt at toxin production may be briefly summarized.

(1) Each of the six strains yielded a filtrate of which 0.1 c.c. killed a "250 gramme" guinea-pig within 48 hours when injected under the skin. Later tests showed that the M.L.D. of the various filtrates approximated to 1/100 c.c. more or less and that a mixture consisting of equal volumes of each gave an M.L.D. of 1/100 c.c. (Table II).

(2) 200 M.L.D.'s of the pooled filtrates when mixed with five units of diphtheria antitoxin and injected subcutaneously produced neither local nor general symptoms in a guinea-pig; a result demonstrating complete neutralization of the toxin by diphtheria antitoxin (Table IV).

(3) The L + dose of the several toxins from the six strains varied from 0.5 c.c. to 1 c.c. (Table V).

(4) Intracutaneous tests of the individual toxins gave skin reactions with amounts corresponding with the relation known to exist between the subcutaneous minimal lethal dose and the intracutaneous minimal reacting dose of diphtheria toxin (1 : 1/500) (Tables III and VI).

These results confirm Capt. Minett's conclusion that the cultures are veritable strains of the diphtheria bacillus. Recent experience indicates that two of them, namely "L" and "D 34," are equal from the point of view of toxigenic ability to the routine diphtheria strains—most of them derivatives of the No. 8 bacillus of Park and Williams—that are used in this laboratory. Thus, later batches of toxin made from strains "L" and "D 34" each gave an M.L.D. of 1/450 c.c. and an L + dose of 0.13 c.c. (Tables VII and VIII).

Cobbett (1900) reported an instance of horse diphtheria which in the following circumstances apparently conveyed the infection to a child: a little girl having fallen ill of diphtheria, Dr A. Mearns Fraser, M.O.H. of Portsmouth, while seeking the source of infection, found that a pony belonging to the child's father was ill with a purulent and sanguineous discharge from its nose. From the nasal mucus Dr Fraser isolated a bacillus morphologically indistinguishable from the diphtheria bacillus; Cobbett to whom the culture was sent proved that it was a true diphtheria bacillus.

Capt. Minett's findings strengthen the views put forward by Cobbett that horse diphtheria is of practical importance in relation to the Public Health and that the occurrence of the disease in horses throws light on the comparative frequency of "normal" antitoxin in their blood.

The discovery of the diphtheria bacillus in superficial septic lesions in the horse apart from specific infection of the nasal mucosa is paralleled by recent

observations on the human subject. Thus, there is growing evidence that in Man the *B. diphtheriae* is apt to be associated with a variety of chronic skin lesions or may become implanted upon cutaneous or subcutaneous lesions already infected with pyogenic bacteria, for example, war wounds and friction sores. Martin (1917) isolated virulent diphtheria bacilli from sores of this kind which were refractory to the usual methods of treatment. It is significant that they occurred in men of the Australian Light Horse. Dr Martin informs me that similar observations were made afterwards by others in Egypt and Palestine.

Capt. Minett's observations suggest that reciprocal contagion of horse and human diphtheria happens more frequently than has been hitherto suspected. Inquiries undertaken with this probability in mind when the circumstances of infection are obscure, may lead to the detection and prevention of cases of diphtheritic infection; and may indicate effective treatment with antitoxic serum.

Table I.

Designation, source and date of isolation of 6 out of 12 cultures obtained from septic lesions in horses by Capt. F. C. Minett, R.A.V.C.

Designation	Source	Date of isolation
L	Pus from suspected case of ulcerative lymphangitis in horse at A.	2. vi. 1919
G	" " " " " " " " at M.H.	Prior to 24. vi. 1918
H	" " " " " " " " at C.	6. ii. 1919
O	" " " " " " " " mule at L	6. ix. 1919
D 16	" " " " " " " " horse at O.	28. x. 1919
D 34	Swab from trephine opening in check of horse at W.	17. x. 1920

Tables II, III, IV, V, VI.

Tests on guinea-pigs weighing *circa* 250 grammes with toxins derived from the 6 strains; cultures inoculated on 21. i. 21 and filtered on 28. i. 21.

Table II. *Subcutaneous M.L.D.*

Date of test	Culture	Dose	Day of death
4. ii. 1921	L	1/100 c.c.	3rd
"	G	"	8th
"	H	"	6th
"	O	"	9th
"	D 16	"	—
"	D 34	"	6th
10. ii. 1921	Mixture of equal volumes of 6 toxins	"	4th

NOTE. The animals that died from the subcutaneous inoculation of filtered cultures and of which the deaths are recorded in this and the following tables were examined postmortem and were found to present the appearances characteristic of diphtheria toxæmia in guinea-pigs.

Table III. *Intracutaneous test.*

Date of test	Culture	Dose	Cutaneous reaction on 4th day	Dose	Cutaneous reaction on 4th day
3. ii. 1921	L	1/25,000 c.c.	necrosis + +	1/50,000 c.c.	necrosis +
"	G	"	" + +	"	" tr
"	H	"	" + +	"	" + +
"	O	"	" +	"	" tr
"	D 16	"	" +	"	" tr
"	D 34	"	" +	"	" tr

NOTE. tr = trace: + = slight: + + = definite.

*Diphtheria Bacillus*Table IV. *Neutralization of mixture of toxins of 6 strains by diphtheria antitoxin.*

Date of test	Dose	Result
10. ii. 1921	2 c.c. of mixed toxins = 200 M.L.D.'s + 5 units of diphtheria anti-toxin	No local reaction nor general symptoms; progressive increase in weight

Table V. *Subcutaneous test of L + dose of toxins.*

Date of test	Culture	L + dose
14. ii. 1921	L	0.75 c.c.
19. ii. 1921	G	0.75 c.c.
"	H	0.75 c.c. nearly*
"	O	0.75 c.c. nearly†
17. ii. 1921	D 16	1.0 c.c.
21. ii. 1921	D 34	0.5 c.c.

* Death between 5th and 6th day.

† Death between 6th and 7th day.

Table VI. *Intracutaneous test of L + dose of toxin "L."*

Date of test	Culture	Dose	Result
9. ii. 1921	L	1/500 A.U. + 1/2000 c.c. toxin	0
"	"	" 1/1000 c.c. toxin	0
"	"	" 1/666 c.c. toxin	? tr necrosis
"	"	" 1/500 c.c. toxin	necrosis + +

NOTE. A.U. = antitoxin unit.

Tables VII and VIII.

Tests with later toxins derived from strains "L" and "D 34." "L" culture inoculated on 1. iii. 1921 and filtered on 10. iii. 1921; "D 34" culture inoculated on 2. iii. 1921 and filtered on 11. iii. 1921.

Table VII. *Subcutaneous M.L.D.*

Date of test	Culture	M.L.D. of toxin
21. iii. 1921	L	1/450 c.c.
"	D 34	1/450 c.c.

Table VIII. *Tests of L + dose.*

Date of test	Dose	Result with "L" toxin	Result with "D 34" toxin
2. iv. 1921	1 A.U. + 0.2 c.c. toxin	Death within 48 hours	Death within 48 hours
6. iv. 1921	" + 0.15 " "	" "	" "
9. iv. 1921	" + 0.13 " "	" "	Death on 4th day
4. iv. 1921	" + 0.10 " "	No local reaction: g.-pig gained weight	No local reaction: g.-pig gained weight

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THE SPREAD OF BACTERIAL INFECTION¹.

THE POTENTIAL INFECTIVITY OF A SURVIVING MOUSE-POPULATION, AND THEIR RESISTANCE TO SUBSEQUENT EPIDEMICS OF THE SAME DISEASE.

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Director of Institute of Pathology, Charing Cross Hospital.

(With 2 Charts.)

IN a previous communication (1921) experiments have been described showing the effect produced by continuously adding normal mice to a cage, the population of which is infected with bacilli of the group which includes *B. gaertner* and *B. suipestifer*. It was seen that under these circumstances the spread of infection progresses in a series of epidemic waves, and that the survival-time of any batch of mice varies according to the period at which they are introduced to the cage. It was further shown that, if such regular and continuous addition of susceptible animals be persisted in over long periods of time, all the mice will eventually succumb, while, if the addition of new individuals be discontinued, the epidemic will eventually subside, leaving a proportion of survivors who may remain in apparently good health over a considerable period.

In one experiment, in which an infected population was thus kept isolated for 77 days after the last addition had been made to the cage, the 15 survivors were killed and examined post-mortem. From nine of them the organisms which caused the epidemic were recovered. It therefore seemed probable that a condition of equilibrium had been attained between the parasites and their hosts, which might be maintained indefinitely so long as the surviving population was kept isolated from susceptible individuals of the same species. The experiments outlined in the present report were undertaken in order to ascertain how this equilibrium would be affected by the addition to the cage of a relatively large number of normal animals.

Two experiments have so far been carried out on mice infected with *B. gaertner* and *B. suipestifer*. It has already been noted that infection with either of these organisms produces a disease which is indistinguishable from that produced by the other, and that if an epidemic be started by feeding mice on a culture of *B. gaertner*, *B. suipestifer* may be isolated from a high proportion of the animals subsequently dying, either alone or associated with the former organism.

¹ A Report to the Medical Research Council.

Experiment I.

This experiment was carried out on the survivors from an epidemic which has been recorded in the communication already referred to. The original experiment was commenced on May 18th, 1920. The epidemic was well under way by the latter half of June, and the addition of normal mice was discontinued on July 24th. Until that date three normal animals had been daily added to the cage. The course of events from July 2nd onwards is recorded in Chart I. It will be seen that the epidemic had practically come to an end by the middle of September, though one death occurred on the 28th of that month, on which day there were five survivors. No further deaths occurred up to November 12th, and on that date 20 normal mice were introduced into the cage. A study of the chart, in which every shaded square corresponds to the death of one mouse, while the death of each survivor from the earlier epidemic is indicated by an unshaded square marked with a dot, will show that a small group of deaths occurred between November 18th and December 6th. During this period none of the survivors succumbed. There was no satisfactory evidence however that the deaths were the result of the infection under consideration. This lack of evidence may well be due to the fact that several of the dead mice were eaten by their companions, but the cause of death must be regarded as undetermined. For more than a month after December 6th no death occurred in the cage; but on January 12th another outbreak commenced, which resulted in the death of 16 of the 19 surviving mice during the following 31 days. The three remaining animals died during the following six weeks, and the last to succumb was one of the survivors from the original epidemic. Of the 25 mice which died after the addition of the fresh susceptibles, four were eaten by their companions, in two the cause of death was undetermined, while from the remaining 19 *B. suipestifer* was isolated, usually in pure culture. It may be noted that the great majority of the mice dying during the original epidemic yielded cultures of *B. gaertner*, while *B. suipestifer* was isolated from relatively few animals. This variation in the serological type of the prevalent organism in the two outbreaks may well have introduced a disturbing factor.

It appears that the addition of a considerable number of susceptible individuals to a population which has survived an epidemic of disease, leads to the outbreak of a fresh wave of infection. During this second epidemic some or all of the survivors themselves may succumb, but the newcomers suffer first and more severely. This point would be more satisfactorily brought out in this experiment if we could be certain of the cause of the earlier group of deaths. It is however clearly indicated in the experiment next to be described.

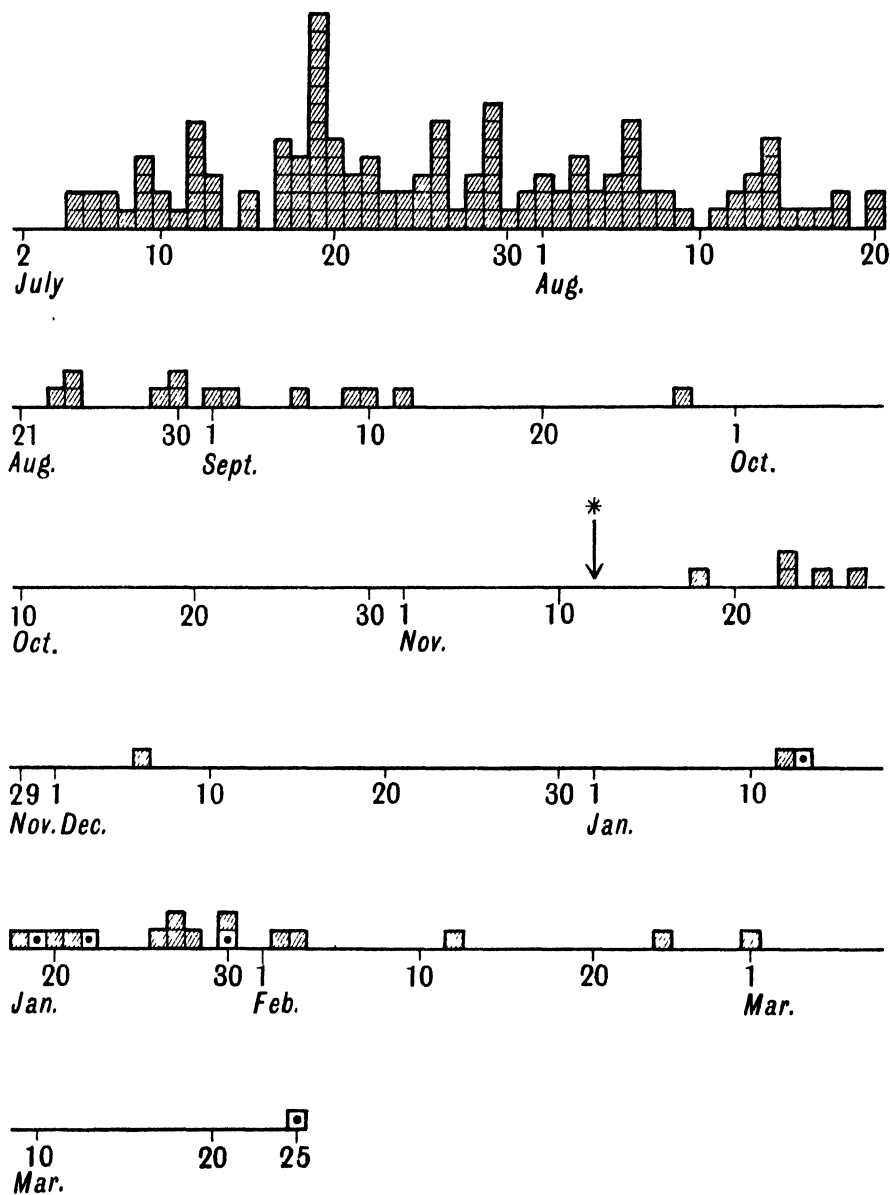


Chart I.

* 20 normal mice added.

Experiment II.

In this experiment advantage was taken of an outbreak among the normal stock, which was probably caused by an accidental spread of infection from the experimental cages. Deaths began to occur on August 14th, 1920, and between that date and August 23rd 26 deaths took place in a number of cages placed together in a large enclosure. From nine of these mice *B. sui-pestifer* was isolated, from eight *B. gaertner*, and from three both types of organism. From the remaining six mice no member of this group was recovered, but the evidence was strongly in favour of their having succumbed to the same infection. On August 23rd there were 124 survivors in these cages. Forty were living in four cages in each of which deaths had occurred. The remaining 84 were distributed among eight cages which had shown no evidence of infection; but since there had been ample opportunity for cage-to-cage infection within the enclosure it is unlikely that they had altogether escaped. These 124 mice were now placed together in a large experimental cage. The subsequent course of events is shown in Chart II which is constructed on the same principle as Chart I. It will be seen that 111 mice succumbed to the epidemic between August 23rd and October 14th. Thirty of these could not be examined post-mortem since they were eaten by their companions. From 13, which were examined post-mortem, no organism of the gaertner-suipestifer group was isolated. From the remaining 68 mice cultures of *B. suipestifer* were obtained from the heart or spleen, or from both situations, usually in pure culture.

During this period two mice had been accidentally killed, so that on October 14th there were 11 survivors. By November 22nd no further deaths had occurred, and on that date 44 normal mice were added to the cage. Only two deaths occurred between November 22nd and January 5th. One of these animals was eaten: in the other the cause of death remained undetermined. On January 6th one mouse died from a typical *B. suipestifer* infection. Another died on the 14th, and a few days later a definite epidemic was established. Between January 14th and February 16th 39 mice died, while during the following month four of the remaining animals succumbed. The other nine mice remained in apparently perfect health for another month, when the experiment was discontinued. From 42 of the 45 mice, which died subsequently to the addition of the fresh susceptibles, *B. suipestifer* was isolated post-mortem. One mouse was eaten by its companions, and in two the cause of death was undetermined. As will be seen from the chart, 24 of the newcomers met their death before the first of the survivors from the original epidemic succumbed to the fresh outbreak of infection. At the time when the experiment was discontinued 39 of the 44 newcomers had died, while four of the 11 survivors from the original epidemic also survived the second.

In these, as in all similar investigations, the value of any conclusions which may be drawn depends largely on the consistency of the evidence obtained in a considerable number of experiments; and the results of those reported here

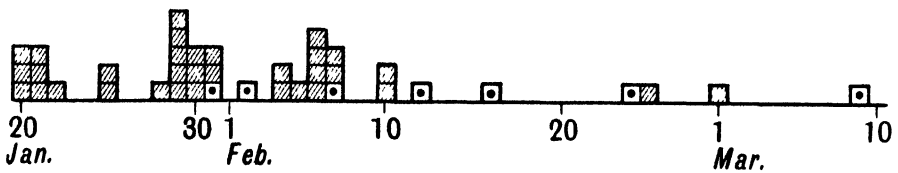
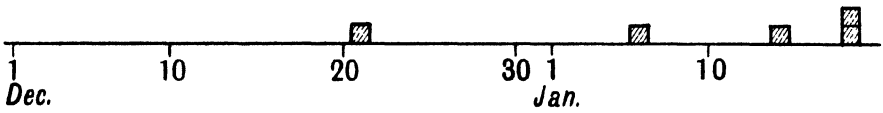
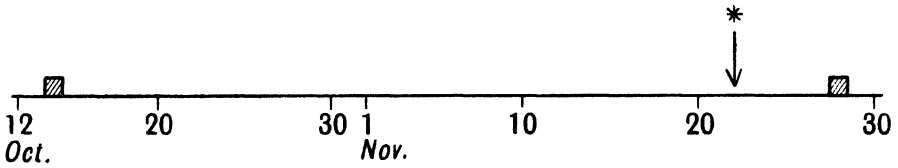
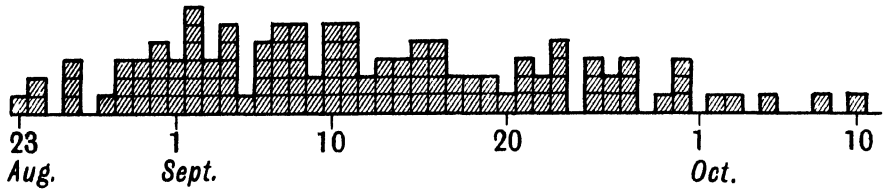


Chart II.

* 44 normal mice added.

must clearly be checked by others carried out along similar lines. Such experiments, however, involve observations carried out over many months, while the course of events in those described above accords well with the facts already elicited.

In the report referred to above it was concluded from the evidence obtained that, if a population which is subjected to an epidemic of bacterial infection be kept isolated, a variable proportion will survive, and that the greater number of these surviving individuals have not escaped infection, but have successfully resisted it. It was suggested that a possible explanation of the continued existence of these survivors in apparent well-being was the establishment of some equilibrium between parasite and host. Such an equilibrium would probably depend upon the infectivity and distribution of the parasite, and the relative immunity of the hosts, either acquired as the result of a mild or atypical infection, or natural to the individuals concerned.

The experiments here reported have shown that such a surviving population may live together in apparent health for a considerable period; and this fact has been confirmed by the observation of several other experimental epidemics. It is further shown that such surviving populations, the individuals of which are apparently non-infective towards one another, possess a quite definite infectivity for fresh susceptibles which are subsequently added to the cage. The considerable period, which elapsed in each experiment between the introduction of these susceptibles and a definite outbreak of the infection concerned, suggests that we are not dealing with an infection of uniform intensity, passed from a healthy carrier to one or more susceptible individuals, but that some process is set in motion, which results in an increase in the infectivity of the parasite, this in its turn giving rise to a fresh wave of mortality among the cage-population.

The fate of the survivors from the original epidemic is of especial interest. It will be seen that they tend definitely to outlive the newcomers to the cage. The fact that these mice, which are immune to the risk of infection from their original companions and able to withstand the earlier stages of the fresh spread of infection among the new arrivals, themselves succumb during the later stages of the epidemic, points strongly to the conclusion that the fresh wave of mortality is associated with a definite increase in the infectivity and virulence of the parasite. Such a variation has been suggested in previous communications (1919, 1921) as an essential factor in the epidemic spread of bacterial infection.

If it be a general law that a state of equilibrium is established in a population which has passed through an epidemic of bacterial disease, rendering the individuals immune to existing conditions, and even to the earlier stages of a fresh spread of infection, but leaving them potentially infective to a second population which has not been subjected to a similar process, we might find an explanation for many puzzling facts in human and animal epidemiology. The evidence is as yet too slight to justify an elaboration of this aspect of the subject, but one instance may perhaps be noted.

The influenzal epidemics of remote islands, and their apparent association with the arrival of a ship from foreign parts, have long formed a fascinating subject for speculation. Hirsch (1881), who it will be remembered sums up against the infectivity of this disease, thus concludes his relation of such outbreaks: "The fact itself can hardly be doubted; while the striking thing appears to me to be that the strangers themselves, in all the cases, have remained exempt or almost exempt from the epidemic."

CONCLUSIONS.

So far at least as the spread of enteric infection among mice is concerned, the experiments here described suggest that:

(1) A population which has passed through an epidemic of bacterial infection may, when completely segregated, survive for considerable periods without any fresh outbreak of the disease in question.

(2) In spite of their apparent freedom from the disease, such survivors are potentially infective towards fresh susceptibles of their own species.

(3) Should a fresh outbreak of the disease occur through the accumulation of such susceptible individuals, the relative immunity of these survivors will carry them through the earlier phases of the new epidemic, but they will tend to succumb during its later stages.

I should wish again to express my indebtedness to my colleagues Dr H. B. Weir and Dr G. S. Wilson for their constant help in these investigations.

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AN INQUIRY INTO THE NATURE OF THE SEROLOGICAL DIFFERENCES EXHIBITED BY DIFFERENT CULTURES OF A BACTERIAL SPECIES (*B. TYPHOSUS*)¹.

By A. DUNCAN GARDNER AND E. W. AINLEY WALKER.

(From the Department of Pathology, University of Oxford.)

(With 5 Charts.)

A Report to the Medical Research Council.

IN a previous communication² one of us showed that certain strains of *B. typhosus* presented marked serological differences. The experiments, to which we now propose to make further reference, were conducted in the following manner.

Four strains were taken, and a quantity of agglutinable culture of each strain was prepared and standardised for opacity. These cultures were then used in making parallel daily tests of the serum of a rabbit, successively immunised against three of the strains by means of single intravenous inoculations of their standardised cultures. The general character of the results was in agreement with conclusions reached by one of us in previous work (1899-1901)³, but showed still greater serological differences between the strains. Thus, the evidence obtained separated the four strains into two serological groups, two strains falling into each group.

The three successive inoculations (on days 1, 8 and 16) were made with three different strains, and after each inoculation the titre of the serum rose for the homologous group to a point very greatly higher than its titre for the strains in the heterologous group. These observations have already been figured for the first 14 days of the experiment (*loc. cit.*). But the whole data obtained during 25 days are now recorded in standard agglutination units (Table I), and the readings for days 7, 13, 18 and 25, which illustrate the points of special interest, are charted in the accompanying diagram (Chart 1).

The cultures used are indicated as T.E., T.L., T.O., and T.T. The rabbit was inoculated intravenously on day 1 with 0.1 c.c. (25 million approximately) of T.L., on day 8 with an equal dose of T.T., and on day 16 with the same dose of T.O. The tests of the serum were put up by one of us, and were read by the other (A. D. G.) who was kept in ignorance of the details of the test before him until the readings had been made and recorded.

¹ Received February 17, 1921.

² Walker, E. W. Ainley (1918). *Journ. of Hygiene*, xvii. 380.

³ Walker, E. W. Ainley (1901). *Journ. of Path. and Bact.* vii. 250.

Table I.

Day	Culture			
	T.E.	T.L.	T.O.	T.T.
1	<14	<14	<14	<14
2	—	—	—	—
3	—	—	—	—
4	65	75	17	14
5	1925	1425	300	345
6	6900	6450	600	570
7	7800	8300	730	600
8	6800	7800	780	570
9	6450	6900	565	513
10	—	—	—	—
11	3450	3850	1090	1290
12	3225	3150	2538	2463
13	3038	3225	6900	7300
14	2960	2920	5600	5000
15	2580	2780	4920	4150
16	2000	1460	2900	3000
17	—	—	—	—
18	2260	1660	2900	4000
19	2000	1360	5540	5000
20	1360	1300	6230	4550
21	1965	1170	9750	9300
22	1695	915	10350	10200
23	—	—	—	—
24	—	—	—	—
25	1650	1000	10580	9950

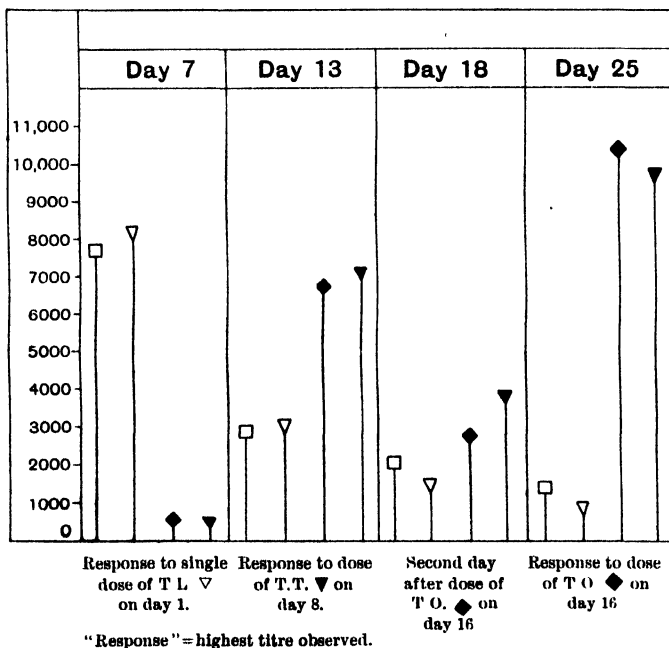


Chart 1.

It is clear from the tabulated results of the experiment just described that T.E. and T.L. may be regarded as constituting one group (Group 1), and T.O. and T.T. as constituting another group (Group 2), which present definite and well-marked serological differences. That fact was sufficient for the purpose in view at the time confirming, as it did, and amplifying the old experiments already referred to, which were also adduced in evidence. No further explanation of the phenomenon was suggested, and its discussion was purposely withheld, partly because it might have distracted attention from the clear and simple issue under examination, but still more because it would have trenched upon the province of another communication dealing with the question of velocity of reaction in relation to agglutination, which one of us (A. D. G.) was then intending shortly to make. Circumstances subsequently prevented that intention from being carried out, and misunderstandings in regard to the interpretation of these experimental results, and doubts as to their accuracy, have since arisen.

On the one hand it has been stated that the phenomenon has never been observed by other workers, and does not occur. On the other hand the observations have been taken to supply an argument against reliance on the technique and methods of interpretation of agglutination tests introduced and developed by Dreyer.

In evidence that the phenomenon already twice described by one of us¹ does actually occur, we may state at once that in the course of the experiments to be described below we have recorded the following observations:

1. We have reproduced the phenomenon in question twice over with the identical material of cultures T.E. and T.O. (of which a supply fortunately remained over in our possession), using two fresh rabbits. One of these was first inoculated with T.E. and subsequently with T.O., while the other was first inoculated with T.O. and then with T.E.

2. We have prepared two other cultures of *B. typhosus* which exhibit similar serological differences, and have demonstrated this difference ten times in experiments on four other rabbits.

3. Finally we have found that the sera prepared by the inoculation of one member, or both members in succession, of either of these pairs of cultures exhibit the same serological difference with the other pair as they exhibit with their own pair of cultures.

The reliability of the observations being thus placed beyond reasonable doubt, the question next arises whether this phenomenon and its interpretation should diminish or increase our confidence in the reliability of Dreyer's method of performing agglutination tests and interpreting their results. On this point we will only offer one comment at present, namely that we are quite unable to see how, by any other method known to us, the occurrence of

¹ Walker, E. W. Ainley (1918). *Journ. of Hygiene*, xvii. 380; Walker, E. W. Ainley (1901). *Journ. of Path. and Bact.* vii. 250.

the phenomenon could have been proved so indubitably, and the observation freed from problematical and purely speculative hypotheses.

In the communication already mentioned as being questioned and misunderstood the following statement was made: "It is not any part of my present intention to discuss the meaning of the very striking differences in agglutinability exhibited by these four strains of *B. typhosus* under the experimental conditions just described. They are associated with other interesting and well-marked characteristics which do not bear immediately upon the question at issue." These other characters will shortly be referred to, but before this is done it appears to be essential to reach a clear understanding as to what is meant by the term "strain," since we have been informed that its use has been misunderstood. In making use of this term we ourselves do not assume at the outset anything in reference to the cultures so spoken of, except that they have been obtained from different sources, derived by different methods of cultivation, or selected for any purpose from a single culture. This we believe is in agreement with ordinary usage. We do *not* assume that they are necessarily in any way different from each other, and still less that they present any *permanent* differences. Whether differences in respect of particular characters exist or not, is always a matter for investigation in each particular case.

That permanent differences exist between different examples of particular well-recognised pathogenetic bacteria we are always inclined to disbelieve until strong evidence to the contrary is forthcoming, since the experience gained in the study of certain forms (streptococcus, meningococcus) seems to show that there is sometimes a tendency for authors to assume permanence and specific value for differences which turn out to be shifting in character, or even interchangeable. But where such differences are proved to exist the forms concerned will constitute species or varieties, and will not be spoken of as strains.

Of the typhoid strains T.E., T.L., T.O. and T.T., the experiment already quoted shows that the first two, T.E. and T.L., were not greatly different from each other serologically; but they differed serologically from T.O. and T.T., which again were serologically similar to each other. Nevertheless, one of us (A. D. G.) was able to obtain quite easily from the strain T.O.—the only one then tried—subcultures which were serologically similar to T.E. and T.L.

It is a common observation that the cultures of any given bacterium obtained from different sources may differ to a greater or less extent in some of their characters. The individuals which compose the population—as we may call it—of a bacterial culture are not identical, but differ among themselves. So do the progeny of any single bacterium. Accordingly, whether that bacterium is propagated in the ordinary way, or through individual colonies by a succession of platings, its variable characters will sooner or later exhibit their variations in the course of repeated subculture. Still more may this be seen if the micro-organism is subjected to more or less diverse environmental

conditions. And this has inevitably been the case with strains obtained from different sources.

Since the population of a bacterial culture is composed of individuals which differ among themselves it is quite possible to demonstrate indications of diversity among the primary subcultures of a series of colonies obtained by plating out from a single colony. And it would theoretically be possible to determine the limits of variation exhibited by the progeny of a single bacterium by examining them all in the primary subcultures of all the colonies on a series of plates. But whether the variations exhibited by strains and variants obtained either from different sources or by intentionally subjecting the micro-organism to an altered environment are greater than the differences existing among the population of a single colony cannot at present be conclusively determined. Yet it is of interest that in the experiments recorded below we found that two colonies selected from a particular plating of a population of *B. typhosus* yielded on subculture in bouillon two cultures, T.M., and T.Non. differing as widely serologically as any two of the strains chosen from different sources. This result is in complete and confirmatory agreement with the statement repeatedly made to us by Professor Dreyer, that he has never in his own experience seen greater differences between so-called strains of these organisms than may be found in different cultures of the same strain.

DESCRIPTION OF EXPERIMENTS: FIRST SERIES.

The characters which distinguished the strains T.O. and T.T. from T.L. and T.E. were non-motility and relative inagglutinability with ordinary standard typhoid agglutinating serum. The association of relative inagglutinability with non-motility was, in our own work, first noted by A. D. G., but we subsequently became acquainted with the fact that it had been observed by Malvoz and studied by Nicolle and Ternel, and later by Theobald Smith. To their investigations and conclusions we shall have occasion to return.

The experiments now to be recorded were conducted in the following manner. Four rabbits (B 1, B 2, B 3, and B 4) were used in the preparation of serums for investigation of the cultures T.M. and T.Non. The latter were formolised bouillon cultures, each derived from a single colony picked off the same plating of one of his stock *B. typhosus* cultures (T.E.) by A. D. G. T.Non. was from a colony which, when grown for 24 hours in bouillon, gave rise to a population apparently devoid of motile elements. A similar population derived from the T.M. colony, consisted almost entirely of actively motile bacilli. The cultures were diluted with formolised normal saline solution to standard opacity precisely as in the preparation of standardised agglutinable cultures of *B. typhosus*, and the whole of the experiments were carried out with one and the same stock suspension of each type of bacillus. Two other rabbits (B 5 and B 6) were used in preparing serums for the re-examination of the cultures T.E. and T.O., of which a quantity remained over in cold storage from the experiments of February and March 1918 recorded above.

The rabbits were immunised by intravenous inoculations of the formolised cultures, each inoculation consisting of 1 c.c. of the culture concerned, containing approximately 250–300 million bacilli. Thereafter the animals were bled (from the ear) for the preparation of serum on the eighth day, counting the day of inoculation as the first day.

Somewhere about 30 c.c. of blood were taken at a bleeding, and the serum was preserved by the addition of 0.3 per cent. of phenol. After each bleeding a period was allowed to elapse before the next inoculation; and before such inoculation was made a sample of blood (3 or 4 c.c.) was collected, and serum prepared, in order to determine by agglutination tests the starting point of the reaction to the second or third dose of antigen.

The rabbits used were all over 2000 grms. in weight, with an average of 2490 grms., and they remained in good condition throughout the experiments. The scheme of the inoculations is shown in Tables II and III.

Table II.

Rabbit	1st inoculation day 1 antigen	2nd inoculation day 15 antigen	3rd inoculation day 42 antigen
B 1	T.M.	T.M.	T.Non.
B 2	T.M.	T.Non.	0
B 3	T.Non.	T.M.	0
B 4	T.Non.	T.Non.	T.M.

T.M. = motile form, T.Non. = non-motile form, isolated from a population of T.E.

Table III.

Rabbit	1st inoculation day 1 antigen	2nd inoculation day 19 antigen
B 5	T.E.	T.O.
B 6	T.O.	T.E.

T.E. = motile strain, T.O. = non-motile strain.

It will be seen that each rabbit was immunised first with one dose (B 2, B 3, B 5, B 6), or with two successive doses (B 1, B 4) of one of a pair of typhoid cultures, and that subsequently it received a single dose of the other member of the same pair of cultures. And each pair of cultures consisted of a motile and a non-motile form of micro-organism.

The bleedings made one week after each inoculation, and the small sample of blood taken immediately before giving a second or third inoculation, provided us with a total of 22 serums. These serums were tested out in the usual way with Dreyer's technique against their own pair of cultures. Furthermore the serums of rabbits B 5 and B 6 (prepared with T.E. and T.O.) were tested also against cultures T.M. and T.Non.; and a chosen four of the serums of the first series of rabbits prepared with T.M. and T.Non. were tested against T.E. and T.O. as well as against their own cultures.

The results obtained are given in Table IV as end-point readings, after the tubes had remained in a water bath at 51–53° C. for two hours, and subse-

quently at room temperature for 24 hours. The figures in the table represent the highest dilution in which agglutination could be detected naked-eye after the lapse of this period. Preliminary readings were also taken in the usual way at the end of the period of incubation in the water bath. But, owing to the slow rate of flocculation of the non-motile cultures, the 24-hour readings seemed to give a fairer and more satisfactory basis of comparison, and are therefore alone made use of here. The series of dilutions employed was 1 in 25, 50, 125, 250, 500, 1000, 2500, 5000, 10,000, 20,000, 50,000 and 100,000.

Table IV.

Rabbit	Serum	Antigen used	Readings with agglutinable culture			
			T.M.	T.Non.	T.E.	T.O.
B 1	After 1st inoculation	T.M.	5,000	2,500	—	—
	Before 2nd	—	12,500	4,000	—	—
	After 2nd	T.M.	20,000	5,000	20,000	5,000
	Before 3rd	—	10,000	1,000	—	—
	After 3rd	T.Non.	7,000	2,500	—	—
B 2	After 1st	T.M.	4,000	1,000	—	—
	Before 2nd	—	5,000	1,000	—	—
	After 2nd	T.Non.	5,000	2,500	—	—
B 3	After 1st	T.Non.	500	5,000	500	5,000
	Before 2nd	—	500	2,500	—	—
	After 2nd	T.M.	10,000	2,500	20,000	10,000
B 4	After 1st	T.Non.	125	2,500	—	—
	Before 2nd	—	300	2,500	—	—
	After 2nd	T.Non.	700	5,000	1,000	20,000
	Before 3rd	—	500	2,500	—	—
	After 3rd	T.M.	10,000	5,000	—	—
B 5	After 1st	T.E.	2,500	1,000	2,500	1,000
	Before 2nd	—	2,500	500	2,500	500
	After 2nd	T.O.	2,500	10,000	2,500	10,000
B 6	After 1st	T.O.	50	2,500	50	2,500
	Before 2nd	—	25	1,000	50	1,000
	After 2nd	T.E.	10,000	10,000	10,000	10,000

NOTE: A dash in a column of readings means that the serum in question was not tested on the culture concerned. There was not enough material remaining of T.E. and T.O. to carry out the whole series of determinations

EXPERIMENTAL RESULTS.

In reading the table it will be noted that the end-point readings of T.E. and T.O. often show an absolute coincidence with those of T.M. and T.Non. respectively. The coincidences would doubtless have been less absolute had a more finely graded series of dilutions been employed. For instance if two cultures both give some kind of "trace" at 1 in 5000 with a particular serum, and nil at 1 in 10,000, it is possible that the actual end-point of the one might be at 1 in 6000, and of the other at 1 in 8000. But the pursuit of finer distinctions was not material to the present investigation.

The results are exhibited graphically in Charts 2, 3 and 4. An examination of these charts and of Table IV brings out the following points:

1. Both "motile" (T.M. and T.E.) and "non-motile" (T.Non. and T.O.) types of *B. typhosus* give rise on inoculation, in all the animals used, to the production of serums which agglutinate to a greater or less degree both types of bacillus.

2. T.M. and T.Non. present a marked "serological difference." In each of the four rabbits used the serum obtained after a single inoculation, or after two inoculations of the same antigen, acts much more strongly on the homologous bacillus than on the heterologous type. The difference ranges from double up to as much as twenty-fold.

3. T.E. and T.O. show a similar "serological difference," the range in this case running to fifty-fold.

4. T.M. and T.E. exhibit a completely homologous serological character, and the same is true of T.Non. and T.O. For wherever T.M. is the more highly agglutinated of its pair, T.E. presents a similar relation to T.O., and the converse holds equally good. But T.M. and T.E. run together, as also do T.Non. and T.O.

5. In every case where a non-motile type of bacillus was used as antigen in the production of a given serum, the "serological difference" between the types is distinctly greater than where a "motile" antigen was employed.

6. When, after one (or two) inoculations with one type of bacillus (motile or non-motile as the case may be), a rabbit is inoculated with the other type of bacillus, its serum exhibits striking changes in relative agglutinating power. The titre always shows a notable increase for the antigen last injected. For the other antigen the naturally occurring fall frequently continues, though it may be arrested or even slightly reversed. But in any case the ratio of the titres is always completely changed, and this reversal or "cross-over" is the most striking feature of the charts.

We are of opinion that the phenomena just described must be taken into consideration in all observations of serological differences before these are interpreted as affording satisfactory proof of the existence of permanent varieties within a species of bacteria.

The Agglutinative Characteristics of the two Types.

Not only do these two types of *B. typhosus* exhibit the agglutinogenic differences just described, but they also present quite definite and constant differences in their manner and rate of clumping. The motile type forms large, fluffy flocculi in the stronger dilutions, and shows rapidly descending gradation in the size of the clumps as the end-point is approached. The reaction is in general nearly complete at the end of two hours in the water bath.

The non-motile type on the other hand forms small, compact and granular clumps, very similar to those formed by dysentery bacilli. There may be no reading higher than "trace" at the end of two hours incubation in a series of

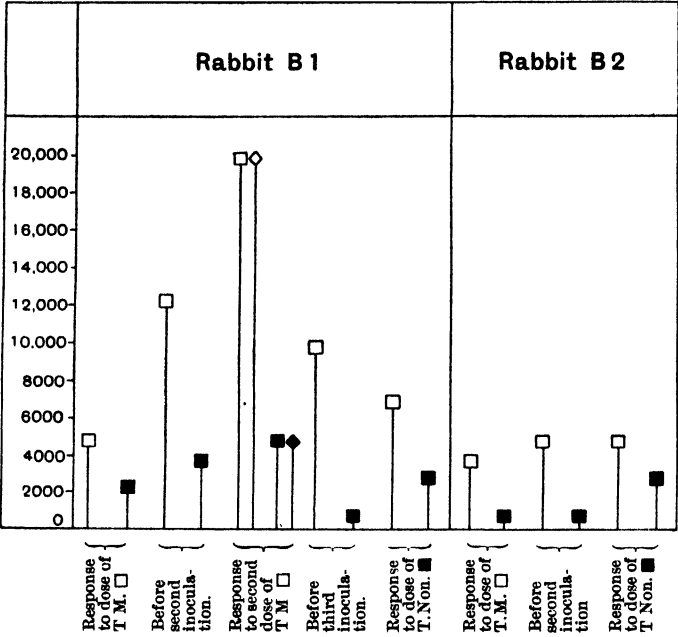


Chart 2.

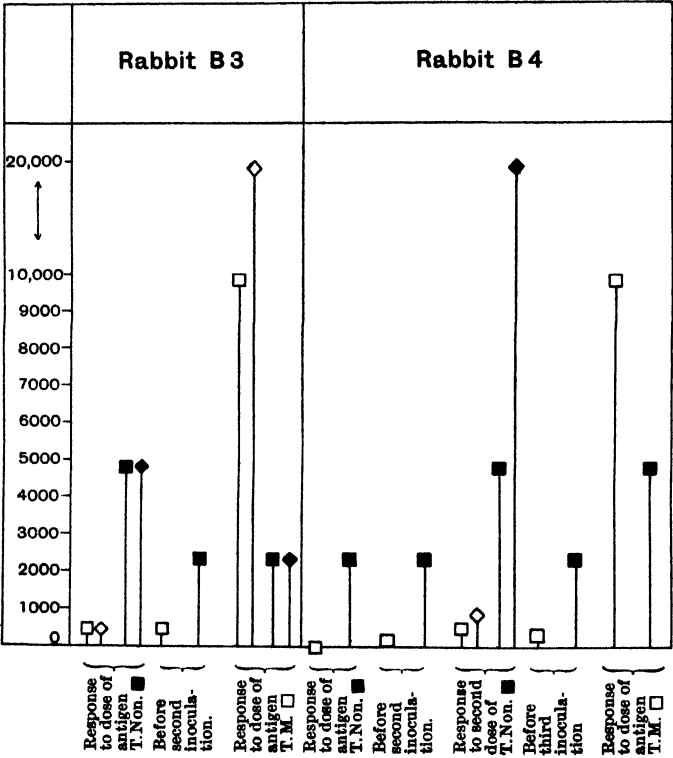


Chart 3.

six or seven dilutions, all showing agglutination, though, after 24 hours, readings approaching "total" appear in the stronger dilutions from the coalescence and sedimentation of the small clumps.

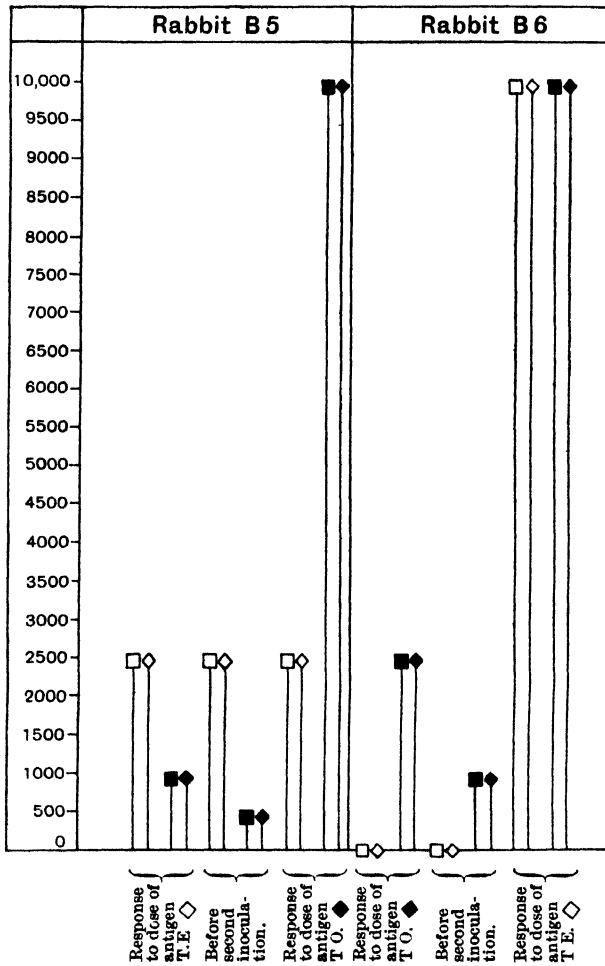


Chart 4.

DESCRIPTION OF EXPERIMENTS AND RESULTS: SECOND SERIES.

When the experiments described above had been completed, and the results worked out, it was suggested to us by Professor Dreyer that some further light might be thrown on the nature of the phenomena observed by conducting a series of experiments with *washed* bacilli. Accordingly a portion of each of the suspensions T.M. and T.Non. was centrifugalised to deposit its bacteria, and the clear supernatant fluid was removed¹. The bacteria thus

¹ In agreement with the fact that T.Non. deposited its bacilli on standing much more quickly (in two or three days) than T.M., it required much less centrifugalisation than the latter to obtain a complete separation of its bacilli.

separated were washed four times with successive quantities of normal saline solution in the usual manner, and were finally suspended in a volume of 0.1 per cent. formalised normal saline solution equal to the original volume of the bacterial suspension.

These suspensions of washed bacilli were used in immunising two pairs of rabbits (B 7 and 8, and B 9 and 10). B 7 and 8 were treated with T.M. and B 9 and 10 with T.Non. Each rabbit received intravenously an inoculation of 1 c.c of the appropriate bacterial suspension on day 1 and day 9 of the experiment, and was bled for the preparation of serum on day 16. The serums were tested out against T.M., T.Non., T.E., and T.O. The readings obtained are given in Table V, and charted in Chart 5.

Table V.

Rabbit	Inoculation	Culture agglutinated	"Titre"	Culture agglutinated	"Titre"
B 7	Washed	T.M.	17000	T.E.	18000
	T.Motile	T.Non.	2500	T.O.	5000
B 8	Washed	T.M.	3500	T.E.	2500
	T.Motile	T.Non.	2500	T.O.	6000
B 9	Washed	T.M.	50*	T.E.	50*
	T.Non-motile	T.Non.	1000	T.O.	3500
B 10	Washed	T.M.	25*	T.E.	<25*
	T.Non-motile	T.Non.	1000	T.O.	3500

* For significance of these figures see text (Discussion).

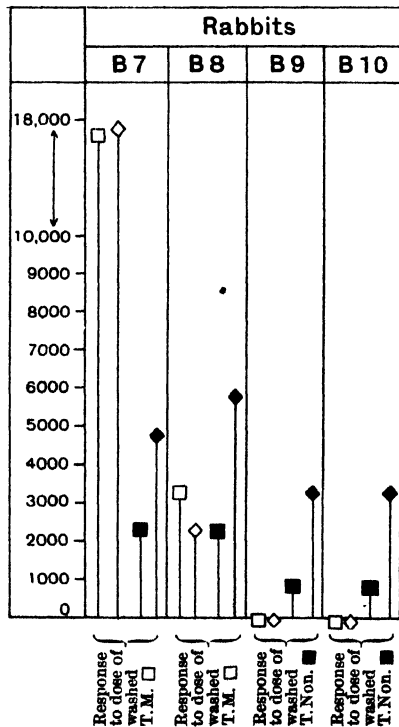


Chart 5.

It will be seen that the washed bacilli gave results, which, though in the main similar to those already described, yet differ in certain important points.

1. In both rabbits treated with the non-motile form the serum produced had so extremely feeble an action on the motile forms that one cannot be certain that the readings exceed the range of possible "normal" agglutination.

This does not, however, constitute an absolute distinction between washed and unwashed bacilli, since in one rabbit (B 6) of three treated with unwashed organisms, the readings were as low as these. Yet the other two rabbits of that series gave quite good agglutination of the motile form. Hence it seems that the difference must be dependent upon the idiosyncrasies of individual rabbits, and not upon differences inherent in the antigens.

2. In one of the two rabbits (B 8), injected with a suspension of the washed motile form, the serum showed relatively high agglutination of the corresponding non-motile suspension (T.Non.), and actually agglutinated the other non-motile suspension (T.O.) in a much higher dilution than either of the motile suspensions (T.M. and T.E.). But the other rabbit (B 7), injected with the same suspension, gave a serum whose action was precisely similar to that of the serums produced by the injection of suspensions of *unwashed* motile forms in the first series of experiments.

DISCUSSION.

The association of inagglutinability with non-motility appears to have been noticed first in *B. typhosus* by Malvoz¹ in 1897. He believed that his experimental treatment of the bacilli removed their flagellated envelope, and that both phenomena were the result of the loss of these structures.

Nicolle and Trenel², 1902, studied variations of agglutinability and of agglutinogenic power of *B. typhosus* and certain other organisms. From one of these which they described as "similar to *B. typhosus*," they produced experimentally motile and non-motile strains, prepared serums with both, and carried out cross-agglutination tests. The "motile" serum agglutinated the motile, but not the non-motile form; whereas the "non-motile" serum agglutinated neither. These results with the non-motile form of the organism stand in contrast with the observations recorded above, and we are inclined to think that a probable explanation of the discrepancy lies in the lack of delicacy of the technique in use at that date. No detailed description of their methods is given by these authors; but the technique which seems to have been in use among French workers at that period was not likely to reveal the minute and very slowly forming clumps of non-motile forms. For example, one or two hours at room temperature would fail to produce any visible clumping.

¹ Malvoz, E. (1897). *Annales de l'Inst. Pasteur*, xi. 582.

² Nicolle, C. and Trenel, M. (1902). *Annales de l'Inst. Pasteur*, xvi. 562.

To reach reliable conclusions concerning the presence or absence of agglutination a technique is required which includes the following features:

1. Heating at the optimum temperature for an adequate period.
2. A further more extended period at room temperature for the completion of the reaction. This is especially important in the case of organisms which do not readily form easily visible clumps.
3. The use of artificial light and a dark background for taking readings; since fine clumping cannot be read properly in any other way.

These requirements are best met, so far as we are aware, by using Dreyer's methods.

Furthermore it is important, so far as possible, to carry out investigations of this character with the same batches of bacterial suspensions throughout the whole series of experiments. For this procedure automatically excludes all question of the relative agglutinability of different batches.

Theobald Smith and Reagh¹, who worked with motile and non-motile forms of "Hog-cholera" bacillus, came to the conclusion that there existed two kinds of agglutinin, the one acting on flagella, the other only on the bacterial bodies. The non-motile form gave rise in animals only to the production of the "body agglutinin," whereas the motile form produced agglutinins for both flagella and bodies. To demonstrate the "body agglutinins" a much higher degree of immunisation was necessary than for the production of "flagella agglutinins."

In our own experiments no higher degree of immunisation was required for the production of agglutinins by the non-motile form. Precisely the same immunising doses were employed in both cases. The non-motile suspension, when unwashed, produced in two animals out of three serums which possessed agglutinating action on the motile form as well as on itself, though their action on the former was relatively low. But both serums, made with the same bacilli after washing, failed to give conclusive evidence of agglutinin production for the motile form.

Benians² obtained an inagglutinable form of dysentery Shiga from a chronic abscess in a guinea-pig, which had been inoculated with "agglutinable" Shiga culture. He found that the inagglutinable form produced no agglutinins for either form. It also failed to absorb agglutinins from an ordinary Shiga serum. But his experimental data are too scanty to justify a final conclusion on these points.

In a short note, Arkwright³ describes the derivation of two forms S. and R. from a number of strains of dysentery, typhoid and several other organisms. The S. form of dysentery Shiga agglutinates in large clumps, the R. form only in fine granules. Both forms agglutinate to the same titre with stock Shiga serum. But when serums are prepared with the S. and R. forms respectively

¹ Smith, Theobald and Reagh, A. L. (1903-4) *Journ. of Med. Research*, x. 89.

² Benians, T. H. C. (1920). *Journ. of Path. and Bact.* xxiii. 171.

³ Arkwright, J. A. (1920). *Journ. of Path. and Bact.* xxiii. Proceedings, 358.

only the homologous culture is agglutinated "in the higher dilutions," and "very little cross-agglutination took place." The data actually given show an appreciable amount of cross-agglutination, in the case of S. serum, and with R. serum show a certain degree, which might have appeared more evident had any test been made below a dilution of 1 in 80.

The author states that somewhat similar results were obtained with *B. typhosus* and with *B. dysenteriae* (Flexner-Y)¹.

The subject of the existence of two different agglutinogenic forms of certain bacteria has also been studied recently by Feiler² in the case of *B. typhosus* and *B. paratyphosus* B.; by Weil and Felix³ for *Proteus* x. 19, and also for certain members of the paratyphoid-enteritis group⁴; by Börnstein⁵ and by Bach⁶ for *Proteus* x. 19 and by Breinl⁷.

The majority of the authors referred to support the view that in these cases two different antigenic factors are in question, which lead to the production of two different agglutinins, but whereas the earlier workers, who investigated motile species only, attributed the differences to the presence or absence of flagella; the discovery by more recent observers of similar phenomena in non-motile species would seem to show that this view is inadequate. Since the two forms are found in motile and non-motile species alike it seems probable that the presence or absence of flagella does not provide the whole explanation, even in the case of motile bacteria.

Our own experiments are not inconsistent with the hypothesis of certain authors that two different agglutinogens exist in variable proportions in the bacterium. On the other hand they do not fully accord with the theory of "body agglutinins" and "flagella agglutinins" as formulated by Theobald Smith and Reagh, since the agglutinins produced in our experiments by the non-motile form, give the same kind of large, fluffy flocculi in suspensions of the motile form as are produced by the homologous serum, though the titre is lower. This fact stands in contrast with the statement of Smith and Reagh that "body agglutinins" are only capable of producing in either form a fine granular clumping.

The evidence at present available does not seem to us to suffice for the formulation of a convincing hypothesis. But if the theory that these bacilli possess two different agglutinogenic properties or factors be accepted provisionally, the following conclusions will follow from our experiments:

1. Every culture of *B. typhosus* examined contains both antigenic factors.
2. The temporary preponderance of one or other agglutinogenic property

¹ Since this Article was sent in to the Medical Research Council for publication Arkwright's full paper has appeared: (1921). *Journ. of Path. and Bact.* xxiv. 36.

² Feiler, M. (1920). *Ztschr. f. Immunitätsf. u. exper. Therapie*, Orig. xxix. 303.

³ Weil, and Felix, A. (1920). *Wien klin. Wochenschr.* xxx. 1509.

⁴ Weil, E. and Felix, A. (1920). *Ztschr. f. Immunitätsf. u. exper. Therapie*, Orig. xxix. 24.

⁵ Börnstein, P. (1920). *Ztschr. f. Hyg. u. Infekt.* xc. 206.

⁶ Bach, F. W. (1920). *Centralbl. f. Bakt. Abt. I* Orig. lxxxiv. 265.

⁷ Breinl, F. (1920). *Ztschr. f. Immunitätsf. u. exper. Therapie*, Orig. xxix. 49.

in the mass of the population of a particular culture determines the serological character of that culture.

3. But the character is only temporary, for it is possible to select *from a single culture* individuals which will produce populations differing as widely serologically as any two "strains" obtained from different sources, or derived by different methods of cultivation. Moreover, a non-motile culture will, under suitable conditions, always (in our experience) yield eventually a fully motile growth.

4. That these different factors both reside within the bacteria themselves, and that the fine granular clumping of the non-motile culture is *not a precipitin reaction* between serum and culture fluid, are demonstrated by our experiments with the washed bacilli.

5. It is clear that in the motile form, whether unwashed or washed, both properties are always present. They are also present in the unwashed non-motile culture, but conclusive evidence of the presence of the "motile" antigenic factor in the washed non-motile suspension is lacking in our experiments. Yet, since this form will readily give rise to the motile form on subculture, we adhere to the view that each individual bacillus, whether in the motile or non-motile phase, possesses both potentialities.

Certain questions affecting the significance and interpretation of these phenomena are under further investigation. Meanwhile the bearing of these observations on the practice of standardising agglutinable cultures must now be considered briefly. It is clear that for the preparation of such cultures it is necessary to use the readily agglutinable form of the bacillus. And in Dreyer's *Directions for the Preparation of Standard Agglutinable Cultures*, it is laid down that repeated subculture in broth should be carried out, for the purpose of increasing the agglutinability of the bacillus. Our experience suggests that this treatment, which almost always gives a satisfactory result, acts by encouraging the multiplication of highly motile forms, which, as we have seen, are more readily agglutinable than elements deficient in motility.

Temporarily non-motile and inagglutinable typhoid bacilli are not infrequently found in the blood or stools of typhoid cases; and although there is no direct evidence on the point, it is conceivable that cases can occur in which the infecting agent is present exclusively in the non-motile phase. If this were so in any particular case, the patient's serum would correspond with our "non-motile" artificial serums, and its agglutination of the motile form (*i.e.* of ordinary good or standard cultures) would be of low grade, or might even be entirely absent.

A case of this kind, if it exhibited the classical symptoms, but yielded no specific bacillus to cultural tests, and if the serum failed to agglutinate reliable cultures of typhoid and paratyphoid bacilli, would be classed as clinical enteric, though bacteriologically and serologically negative. We should like to suggest that it would be well in such rare cases to test the serum with a suspension prepared from a culture of non-motile typhoid bacilli, as well as with cultures of the Gaertner group, which we now know can also cause "clinical enteric."

A STUDY OF THE BACTERIOLOGICAL EXAMINATION OF GRADE "A" (CERT.) MILK¹.

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THE following paper contains a study of the results of bacteriological examinations of samples of Grade "A" (Certified) milk, some of which were 24 hours old and others 30 hours old at the time of examination. This work was started in 1916 and is still being carried on. Some of the results have already been published². For purposes of comparison, these results, together with further series, are included in the present paper.

CONDITIONS OF MILK PRODUCTION AT THE FARM AND CARRIAGE BY RAIL.

The samples of milk were derived from Farms I, II, III and IV.

All these farms are equipped for clean milk production since they possess water, light, steam, covered milking pails and the other necessary dairy appliances.

Farms I, II and III have special sheds for milking, but Farm IV is without special provision in this respect.

The milk was cooled to temperatures which varied between 40° F. and 66° F. on Farm I, between 50° F. and 66° F. on Farm II, 30° F. and 68° F. on Farm III, and 43° F. and 61° F. on Farm IV.

It was then bottled and sample (pint or quart) bottles were taken at random from the bulk and sent each week to the laboratory, in ventilated boxes, by rail. No attempt was made to keep them cool on the journey with the result that the temperatures on arrival at the laboratory varied between 36° F. and 76° F.

In one series the age of the milk was 24 hours, and in a second series 30 hours, on arrival at the laboratory.

The fact that the bulk was so small, allowed the milk to be readily affected by temperature changes and at certain periods of the year the temperatures of cooling and during transit were very unfavourable.

¹ The expenses of this work were in part defrayed by the Nestle and Anglo-Swiss Milk Co.

² Freear, Buckley and Stenhouse Williams (1919), *A Study of Two Types of Commercial Milk*. Cambridge Univ. Press.

BACTERIOLOGICAL EXAMINATIONS.

All the samples were examined by plating dilutions from 1/10 c.c. to 1/10,000 c.c. of the well mixed milk on neutral whey agar plates, which were incubated for five days at 22° C. and then counted. Lactose fermentation tests were also carried out by inoculating quantities of milk varying from 1 c.c. to 1/10,000 c.c. into litmus lactose peptone water and incubating at 37° C.

The presence or absence of gas in the Durham's tubes was noted on the fifth day.

STANDARDS OF COMPARISON.

The results of the bacteriological examinations have been considered in relation to the American standard of 10,000 bacteria per 1 c.c. of milk and also in relation to the 30,000 standard which has been adopted in this country. The lactose fermentation tests have been included as they yield useful information, a fact which will appear later.

RESULTS OF THE BACTERIOLOGICAL EXAMINATIONS OF 201 WEEKLY SAMPLES FROM FARMS I, II AND IV AT THE END OF 24 HOURS.

Table I shows that 201 samples have been examined, 82 of these came from Farm I between November, 1916 and September, 1918, 77 from Farm II between April, 1919 and December, 1920, and 42 from Farm IV between February, 1920 and January, 1921.

Results in comparison with the 10,000 standard.

When the results of the bacteriological examinations are considered it is seen that two or 2.4 per cent. of the samples from Farm I showed counts which were above 10,000 and that seven or 8.5 per cent. produced acid and gas when inoculated into litmus lactose peptone water in quantities of 1 c.c. or less.

Three or 3.7 per cent. of the samples from Farm II gave counts of more than 10,000 and 20 or 26 per cent. gave rise to the production of acid and gas in lactose peptone water.

Seven or 16.6 per cent. of the samples from Farm IV were above the 10,000 standard and 14 or 33.3 per cent. showed acid and gas.

When the results from Farms I, II and IV are combined it is seen that 201 samples have been examined, and that 12 or 6 per cent. of them have failed to maintain the 10,000 standard and that 41 or 20.6 per cent. have contained organisms capable of fermenting lactose with the production of acid and gas.

Results in comparison with the 30,000 standard.

In this country a maximum of 30,000 bacteria per 1 c.c. has been adopted as the standard. It was thought advisable, therefore, to consider the above results in relation to that standard.

In Table I the results are tabulated in this form. The table shows that the

percentages of occasions when the bacterial counts from Farms I, II and IV rose above 30,000 were 2·4, 1·3 and 9·5.

Table 1. *Milk 24 hours old.*

10,000 standard.					
Farm	No. of samples	No. of counts above 10,000	Percentage of counts above 10,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	82	2	2·44	7	8·5
II	77	3	3·7	20	26·0
IV	42	7	16·6	14	33·3
I, II and IV	201	12	6	41	20·6

30,000 standard.					
Farm	No. of samples	No. of counts above 30,000	Percentage of counts above 30,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	82	2	2·44	7	8·5
II	77	1	1·3	20	26
IV	42	4	9·5	14	33·3
I, II and IV	201	7	3·5	41	20·6

When the results were combined the percentage of failure to maintain the standard was 3·5.

COMPARISON OF RESULTS AT THE END OF 24 HOURS.

When the combined results are considered in relation to this standard and are compared with the results on the 10,000 basis it is seen that the lower standard gives an advantage of 2·5 per cent.

It is of interest to note that the lower standard is of no advantage to Farm I but that Farms II and IV benefit to the extent of 2·4 per cent. and 7·1 per cent. The explanation lies in the fact that the bacterial counts from Farm I have always been well below the 10,000 limit except upon the two occasions recorded, when they were above 30,000, whilst Farms II and IV did not exhibit so uniform a standard of purity.

Clear evidence of this is furnished by the fact that 8·5 per cent. of the samples from Farm I contained lactose fermenting organisms as compared with 26 per cent. and 33 per cent. in the cases of Farms II and IV.

The value of the lactose fermentation test as a control of the count is demonstrated, since Farm II possessed all the mechanical appliances found on Farm I and the bacterial counts lay very close together.

The lactose fermentation tests, however, showed that there was a difference in the quality of the labour. Indeed, it would appear that when a certain skill has been attained this test is a very valuable aid in judging the quality of that skill.

The absence of a milking shed on Farm IV makes it difficult to decide the exact influence of labour in this case, but from the work which we have carried

out in a cowshed which is no better than that on Farm IV we are inclined to the opinion that better results could be obtained with improved labour.

BACTERIOLOGICAL EXAMINATIONS AT THE END OF 30 HOURS.

In view of the fact that the official regulations relating to the sale of Grade "A" (Certified) milk require that it shall reach the consumer within 48 hours of production and that the maximum number of bacteria present at any time during that period shall not be more than 30,000, it became important to determine the bacteriological condition of the milk at ages greater than 24 hours.

A series of experiments was, therefore, set up for the study of such milk at 30 hours old. 219 samples have been examined from Farms I and III.

117 of these were received from Farm I between August 8th, 1918 and December 9th, 1920. Farm III provided two sets of samples ("A" and "B"). In the first instance, from October 23rd, 1919 to January 20th, 1921, 62 samples (called "A" in the Table) were taken from the first milk coming over the cooler. Later, it was decided to examine samples of milk taken at a later stage in the same milking. These are called "B" in the Table and the period of examination extended from March 4th, 1920 to January 20th, 1921.

Results in comparison with the 10,000 standard.

Table II shows the results obtained with these samples when compared with a standard of 10,000 bacteria per 1 c.c.

Table II. *Milk 30 hours old.*

10,000 standard.					
Farm	No. of samples	No. of counts above 10,000	Percentage of counts above 10,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	117	16	13.6	23	19.6
III "A"	62	15	24.2	25	40.3
III "B"	40	10	25.0	22	55.0
I, III "A" and "B"	219	41	18.7	70	32

30,000 standard.					
Farm	No. of samples	No. of counts above 30,000	Percentage of counts above 30,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	117	8	6.8	23	19.6
III "A"	62	6	9.7	25	40.3
III "B"	40	7	17.5	22	55.0
I, III "A" and "B"	219	21	9.6	70	32

Out of 117 samples examined from Farm I, 16 or 13.6 per cent. showed counts above 10,000 and 23 or 19.6 per cent. contained lactose fermenting organisms.

15 or 24.2 per cent. of the 62 "A" samples from Farm III contained more than 10,000 bacteria per 1 c.c. and 25 or 40 per cent. contained organisms which

produced acid and gas in lactose peptone water. 40 "B" samples from Farm III are recorded of which ten or 25 per cent. gave bacterial counts above 10,000 and 22 or 55 per cent. gave acid and gas in litmus lactose peptone water.

When the results from Farms I, III "A" and III "B" are combined, the average error is 18.7 per cent. and one-third of the samples showed the presence of lactose fermenting organisms.

Results in comparison with the 30,000 standard.

Table II shows the results of the examination of milk from these farms, when 30,000 bacteria per 1 c.c. was used as a standard of comparison. From this Table it appears that the liability to failure to maintain the standard when compared with the liability to failure to maintain the 10,000 standard is reduced from 13.6 per cent. to 6.8 per cent. on Farm I, from 24.2 per cent. to 9.7 per cent. on Farm III "A" and from 25 per cent. to 17.5 per cent. on Farm III "B."

COMPARISON OF THE RESULTS AT 24 AND 30 HOURS.

These results are brought out in Table III in which the bacterial contents of the samples, at 24 and 30 hours old, are compared.

Table III. *Comparison of Counts at the end of 24 and 30 hours.*

10,000 standard.						
Farm	Age of milk	No. of samples	No. of counts above 10,000	Percentage of counts above 10,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	24	82	2	2.4	7	8.5
I	30	117	16	13.6	23	19.6
		199	18	9.0		
I, II and IV	24	201	12	6.0	41	20.6
I, III "A" and "B"	30	219	41	18.7	70	32.0
30,000 standard.						
Farm	Age of milk	No. of samples	No. of counts above 30,000	Percentage of counts above 30,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	24	82	2	2.4	7	8.5
I	30	117	8	6.8	23	19.6
		199	10	5		
I, II and IV	24	201	7	3.5	41	20.6
I, III "A" and "B"	30	219	21	9.6	70	32

In order to construct this table it was necessary to adopt a commercial standard of cleanliness. The general excellence of the samples from Farm I over a period of more than four years justifies their use as a basis for comparison. During this time 199 samples have been examined, 82 at 24 hours and 117 at 30 hours old. 9 per cent. of these have been found to be above the 10,000 standard and 5 per cent. above the 30,000 standard.

If the results of the examination of milk from Farm I at the end of 24 and 30 hours be considered in relation to the 10,000 standard it is seen that the liability to failure to maintain the standard is increased from 2.4 per cent. at the end of 24 hours to 13.6 per cent. at the end of 30 hours and the percentage of samples showing acid and gas in litmus peptone water has risen from 8.5 per cent. to 19.6 per cent.

On the 30,000 basis the number of samples which showed counts above the standard increased from 2.4 per cent. at the end of 24 hours to 6.8 per cent. at the end of 30 hours.

It follows, therefore, that, if 30,000 bacteria per 1 c.c. be accepted as the standard, Farm I is liable to an increased error of 4.4 per cent. when the examinations take place at the end of 30 hours.

If, now, the combined figures from Farms I, II, III "A," III "B" and IV be considered, it is seen that 201 samples from Farms I, II and IV were examined when 24 hours old and that 6 per cent. failed to maintain the 10,000 standard.

Further, 219 samples from Farms I, III "A" and III "B" were examined when 30 hours old, and the percentage of failures amounted to 18.7, an increase of 12.7.

When, however, the comparison was made on the 30,000 basis it was found that the percentage of failures to maintain the standard had risen from 3.5 per cent. at the end of 24 hours to 9.6 per cent. at the end of 30 hours, an increase of only 6.1 per cent.

The work which has been carried out demonstrates that the milk from Farm I when 30 hours old is liable to exceed the standard on 25 occasions in the course of a year. If the figures for the combined farms be taken this number rises to 35. These variations would be materially increased if the 10,000 standard were adopted since the rise would amount to 11.2 per cent. in the case of Farm I and 12.7 per cent. in the case of the combined farms.

The adoption of such a standard for official purposes would involve the combined farms in an increased liability to error of nearly 100 per cent.

Table III further shows that the percentages of occasions when lactose fermenting organisms were found rose from 8.5 per cent. to 19.6 per cent. in the case of Farm I and from 20.6 per cent. to 32 per cent. in that of the combined farms, when the time of examination was increased from 24 to 30 hours.

It is clear that as the age of the milk increases, it becomes more difficult to maintain the standard, and the liability to error at the end of 30 hours is already sufficiently serious to raise the question of the reconsideration of the present conditions relating to the handling of the milk. The considered opinion of the Milk Industry is required upon this point.

If the industry is in a position to supply this milk to the consumer within 24 hours of milking, then the present methods of production and carriage, with no attempt at keeping the milk cool on the journey, may perhaps be continued.

If that is impossible, and no guarantee can be given that the milk will reach

the consumer within a period of less than 30 hours, then the whole question of methods for chilling the milk both at the farm and during the journey requires consideration. The figures in Table IV demonstrate that, if the milk were sold within 30 hours, then chilling would be necessary during the months May to September. These are technical problems to which answers are required if our future work is to be of maximum advantage to the industry.

Table IV. *Seasonal Variation in Counts of Certified Milk.*

Farms I and II.

Milk 24 hours old.

Period	Counts under 30,000	Counts over 30,000	Period	Counts under 30,000	Counts over 30,000
Oct.-April	87	1	May-Sept.	72	2

Farms I, III "A" and III "B."

Milk 30 hours old.

Period	Counts under 30,000	Counts over 30,000	Period	Counts under 30,000	Counts over 30,000
Oct.-April	131	4	May-Sept.	88	17

The facts recorded show that milk produced under the best conditions can travel for 24 hours and still maintain a very high standard of bacterial purity. When the time is prolonged beyond this, the danger of error steadily increases.

They further show that it is inadvisable to raise the standard at the present juncture. This is the more important since the successful production of Grade "A" (Certified) milk involves the employment of persons possessing certain technical knowledge, a form of labour which is very limited in this country at the present time.

BOVINE TUBERCULOSIS; THE ETIOLOGICAL SUPPORT OF FAMILY HISTORY.

BY

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THE work contained in the following paper demonstrates that valuable evidence of the relative incidence of bovine and human tuberculosis may probably be obtained by a careful record of the chances of contact infection in each case.

The material dealt with was derived from a central clinic in a large industrial County Borough (Newport, Mon.) and from a series of smaller clinics. Two of the latter were in industrial (coal and iron) districts and three in small country towns containing semi-urban and rural populations. The total population of the area exceeds 200,000. The cases analysed were drawn from records of 4000 individuals personally examined by one of us. Approximately 50 per cent. of these were diagnosed as suffering from tuberculosis in one or other of its various forms, and about 10 per cent., or 400 persons, showed evidences of non-pulmonary tuberculosis. In order that the statistics concerning the latter class might be made as accurate as possible the following precautions were taken:

Doubtful cases and cases of micropoly-adenitis in children, and all cases in which the predominant lesion was pulmonary, and the non-pulmonary forms which were secondary to pulmonary infection, were excluded. Cases presenting lesions which proved on serological or histological investigation to be due to other conditions, *e.g.* syphilis, Hodgkins' disease or lymphosarcoma, have been omitted. Every effort was made to secure that the cases analysed were genuine cases of non-pulmonary tuberculosis requiring treatment.

As a comparison, 250 unselected cases of pulmonary disease, in all of which tubercle bacilli had been found in the sputum, have been analysed.

Table I contains the records of 382 cases of non-pulmonary tuberculosis. Fifty-one of these were cases of tracheo-bronchial adenitis and they have been placed in a separate class from the rest. There remain 331 cases of non-pulmonary tuberculosis. Of these 170 were males and 161 were females. In

every case, as a routine measure, the family history was carefully inquired into at the time of the first examination of the patient. It is probable that the history of the presence of tuberculosis in the family is under-estimated in these statistics, but as all the cases were investigated under similar conditions and without any view to the present publication this error can be assumed to be a constant, and, therefore, although the absolute figures of tuberculous family histories are probably too low, the relative percentages in each class of case are not affected.

Table I.

Lesion	Total	M	F	F H ₀	F H ₁	F H ₂	Percentages			Age Periods						
							F H ₀ %	F H ₁ %	F H ₂ %	0-5	5-10	10-15	15-20	20-25	25-30	Over 30
GLANDS: external groups—chiefly cervical	132	67	65	87	32	13	65.9	24.2	9.9	21	34	29	19	15	6	6
JOINTS:																
Ankle and elbow	13	6	7	10	3	0	66.2	24.3	9.4	1	6	2	1	3	—	—
Hip	33	17	16	17	13	3										
Knee	28	11	17	22	2	4										
Spine	37	17	20	24	12	1	64.8	32.4	2.7	8	8	5	4	8	2	2
Other bones	34	24	10	25	8	1	73.5	23.5	2.9	4	6	12	4	2	2	4
							67.5	26.7	6.3							
Tuberc. Peritonitis	29	19	10	18	6	5	61.8	20.6	17.6	6	11	9	3	—	—	—
Skin	17	7	10	10	7	0	58.9	41.1	0	1	1	3	4	1	4	3
Miliary tuberculosis	8	2	6	4	3	1	50	37.5	12.5	8	—	—	—	—	—	—
Total	331	170	161	217	86	28	65.5	25.9	8.4	53	81	79	48	33	18	17
Tracheo-bronchial GLANDS; hilum tuberculosis	51	35	16	19	25	7	37.3	49	13.7	2	36	13	—	—	—	—
Pulmonary T.B. sputum	250	135	115	126	105	19	50.4	42	7.6	—	1	9	36	47	35	122

Again it should be stated that the higher the age group the less reliable are the figures in these groups as an indication of the actual age incidence of the lesion, since after the age of 15 many of the figures of the later groups should be moved to the left. A large number of the adult cases of non-pulmonary tuberculosis had had the disease for a number of years before they presented themselves for examination at the clinic, and the ages given are those at which they were first examined at the clinic. The age incidences of the non-pulmonary types of tuberculosis do not necessarily represent the age incidence of non-pulmonary forms of tuberculosis in the population, but only the age incidences of the cases of non-pulmonary tuberculosis which attended the clinics. The pulmonary cases on the other hand represent with fair accuracy the age incidence of pulmonary tuberculosis in the population. This is probably because tuberculosis clinics are wrongly regarded as being institutions primarily intended for the treatment of pulmonary types of the disease.

An examination of Table I shows that tuberculous cervical adenitis was the predominant type of non-pulmonary lesion seen at the clinic. 132 of the 331 cases were tuberculous infection of glands of the external groups, chiefly cervical.

Tuberculosis of the joints, spine and of the long bones account for 145 cases, various lesions of the skin for 17, tuberculous peritonitis for 29—with one exception all of the ascitic type—and miliary tuberculosis for eight cases. It is seen, therefore, that certain types of tuberculosis, *e.g.* tuberculous meningitis, were not represented at all in the practice of the clinic and it is certain that only the more severe cases of cervical adenitis were sent for treatment.

The family histories of these cases, as a measure of the opportunities of infection, reveal some striking facts. In order to study these histories they have been divided into three groups:

1. Cases in which no history of tuberculosis in the family was admitted.
2. Cases which gave a history of tuberculosis occurring in a near relation, mother or father, brother or sister, husband or wife.
3. Cases which gave a history of tuberculosis in a more remote relation, grandparents, uncles or aunts.

These three groups have been designated F.H.₀, F.H.₁, F.H.₂. The detailed percentages of each variety of non-pulmonary tuberculosis are set out in the table; it will suffice here to consider the general averages. Of the 331 cases, 217 gave no history of tuberculosis in the family, 86 gave a history of tuberculosis in near relations, and 28 in relations more remote. These numbers expressed as percentages give the following figures: F.H.₀, 65.5 per cent.; F.H.₁, 25.9 per cent.; F.H.₂, 8.4 per cent.

The table shows that the possibility of family contact in the two large groups of glandular and bone and joint cases are very similar, since the F.H.₁ percentages (24.2 per cent.—26.7 per cent.) are very close together.

The numbers of cases which are included under the headings Tuberculous Peritonitis, Skin and Miliary Tuberculosis, and under the sub-headings of Diseases of the Joints are too few to justify any conclusions being drawn from the percentage figures.

It is instructive to compare these figures with those obtained in 250 cases of pulmonary disease all of which showed the presence of tubercle bacilli in the sputum. Beyond insuring that this was the fact (and so placing the question of diagnosis beyond dispute) no sort of selection was employed in choosing these pulmonary cases. Of the 250 pulmonary cases 126 belonged to the class F.H.₀, 105 to the class F.H.₁, and 19 to the class F.H.₂. Expressed as a percentage the F.H.₁ figures amount to 42 per cent. compared with 24.2 per cent. for glands of external groups, 26.7 per cent. for joints and 25.9 per cent. for the 331 cases of "Other Tuberculous Diseases," which are included in the table. With these may be considered 51 cases of children under 15 suffering from active tuberculosis of the tracheo-bronchial glands. Nineteen of these gave no family histories (F.H.₀), 25 gave histories of tuberculosis in near relations (F.H.₁) and seven in remote relations (F.H.₂); expressed as percentages F.H.₀ = 37.3 per cent.; F.H.₁ = 49 per cent.; F.H.₂ = 13.7 per cent. Ten of these cases, all falling in group F.H.₁, were examined as contacts with cases of tuberculosis.

The number of these (tracheo-bronchial gland) cases is small, but on the evidence it would appear that the chances of infection by family contact approach the conditions found in cases of pulmonary tuberculosis.

It is of interest to compare the figures for F.H.₁ and F.H.₂ with the percentages of bovine infection discovered by laboratory methods for each class of lesion.

The following table has been compiled from Cobbett's¹ valuable book which appeared shortly after this clinical material had been analysed, and from a table constructed by A. E. Stanley Griffiths² largely founded upon his own experimental work and that of F. Griffiths and Eastwood. The percentages and the numbers of cases upon which the laboratory conclusions for each type of lesion are founded and the percentage and number of instances of each type of lesion in our series are given in Table II. It will be noted that taking the

Table II.

Lesion	Percentage of Bovine infection at all ages	F.H. ₁ (all ages)	F.H. ₂ (all ages)
*Pulmonary	1.41 % (212 cases)	42 % (250 cases)	7.6 %
†Bronchial glands	5.5 % (18 cases)	49 % (51 cases) (or excluding 10 contacts = F.H. ₁ = 36.5 %)	13.7 %
*Cervical glands	47.05 % (102 cases)	24.2 % (132 cases)	9.9 %
*Bones and joints	19.9 % (392 cases)	26.7 % (145 cases)	6.3 %
†Abdominal tuberculosis	51 % (56 cases) (all ages 6 %)	20.6 % (29 cases) (Tuberculous peritonitis)	17.6 %

* Source—Cobbett, *op. cit.*

† „ —A. S. Griffiths, *loc. cit.*

table as a whole there is shown an inverse proportion between the laboratory percentages for bovine infection in each lesion and our figure for F.H.₁. This extends to the bronchial glands class, if the ten contacts previously mentioned are removed, as it seems reasonable to do, since their inclusion might seem to vitiate the haphazard choice which has been observed throughout.

The only real exception to the inverse relationship is the "Bones and Joints class." The laboratory results give 19.9 per cent. to bovine infection. F.H.₁ for this class is 26.7 per cent. We should have expected a higher bovine percentage.

The figures given suggest that the family histories of cases of tuberculosis, if taken in sufficient number, would constitute valuable collateral evidence of the extent of bovine infection in districts. The lower the figure for F.H.₁ the more likely are the lesions to be of bovine origin. More investigations by tuberculosis officers into the family histories of cases of tuberculosis might

¹ Cobbett, C. L. (1917). *The Causes of Tuberculosis*, Cambridge Public Health Series.

² Griffiths, A. Stanley (1907). *Brit. Journ. Tuberculosis*, XI, No. 4.

prove of great value in indicating the probable extent of bovine tuberculosis in the districts with which the officers are concerned; the extent to which it may vary in one district compared with another; and the relative proportions of cases of human and bovine tuberculosis found in districts of different type, as for example in purely industrial compared with purely agricultural areas.

This paper makes it clear that contact infection is not so common in cases of "Other Tuberculous Diseases" as in pulmonary infections. There must, therefore, be some other factor at work in these cases. The discussion of that factor may be left to a second paper.

ON *BACILLUS COLI* INFECTIONS OF THE URINARY TRACT, ESPECIALLY IN RELATION TO HAEMOLYTIC ORGANISMS.

BY

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CONTENTS.

	PAGE
<i>Introduction</i>	137
Section I. The methods employed in the examination of various strains of <i>Bacillus coli</i> for haemolytic properties	138
„ II. <i>B. coli</i> infections of the urinary tract. (i) Haemolytic and non-haemolytic strains; (ii) Differences between male and female infection; (iii) Cultural properties; (iv) A comparison of the agglutinable properties of the two strains	140
„ III. On the presence of haemolytic strains of <i>B. coli</i> in the faeces	146
„ IV. On the presence of coli agglutinins in normal human sera and in the sera from coli infections	149
„ V. On the immunisation of rabbits with vaccines and live cultures of haemolytic and non-haemolytic strains of <i>B. coli</i>	150
„ VI. Coli agglutinins: (i) Methods employed; (ii) Preparation of antigens; (iii) Saturation experiments	158
<i>Conclusions</i>	162

INTRODUCTION.

THOSE who have studied the literature of *Bacillus coli* infections of the urinary tract, over a period of many years, must be fully conscious that the methods employed in such investigations have been on very similar lines. Bacilli isolated from the urine have been classified on the result of various cultural reactions of which the fermentation of cane sugar and dulcitol appears to occupy the most important position. Animals have been inoculated with various strains of urinary coli, while similar inoculation experiments have been made on animals before and after a mechanical injury to one or both kidneys had been produced.

The question whether *B. coli* infections are "ascending" or "haemic" has given rise to considerable discussion of which an undue proportion has been theoretical. For these reasons we decided to investigate this infective condition by entirely different means, with the hope that we might be able to throw light on the various problems which so far had remained unsolved. In our opinion, however valuable experimental observations on animals may have been or may prove to be in the future as regards *B. coli* infections of the urinary tract, yet this condition must be studied fully on the human subject; further, there are few diseases which offer greater opportunities for combined pathological and clinical investigations.

SECTION I.

The Methods employed in the examination of various strains of *Bacillus coli* for haemolytic properties.

Preparation of media. Tubes containing 5 c.c. of 1 per cent. peptone in 0.5 per cent. and 0.85 per cent. sodium chloride in distilled water were employed throughout.

The human blood was collected by vein puncture in tubes containing 5 per cent. sodium citrate. The blood is well mixed with the citrate, then allowed to stand for about one hour, centrifugalised at high speed, and the supernatant fluid discarded.

Method of testing for haemolysis. Two tubes of each strength sodium chloride in peptone water were inoculated, and 0.1 c.c. of solid red cells were added to one tube of each. The four tubes were then incubated at 37° C. for 24 hours. If haemolysis had occurred, the readings were taken, and 0.1 c.c. of red cells added to the two remaining tubes which were then re-incubated for one hour at 37° C., allowed to stand in the ice-safe over-night, and the degree of haemolysis fully noted. The four tubes were used to obtain a comparison of the degree of haemolysis produced in 0.5 per cent. and 0.85 per cent. sodium chloride, when red cells were added at the time of inoculation and after the bacillus had grown for 24 hours. Control tubes with red cells were employed in each experiment. In reading the degree of haemolysis, the tubes must be slightly shaken when necessary to allow the haemoglobin to diffuse through the medium.

The gradations of haemolysis are recorded as "Trace," "Marked," "Incomplete" and "Complete": a trace is slight tingeing of the media above the red cells, and represents an average of 60 per cent. haemoglobin on the Oliver scale: "Marked" is distinct colouration of the whole media and represents an average of 160 per cent. haemoglobin: "Incomplete" is haemolysis of nearly all the red cells, about 260 per cent. average: "Complete" is haemolysis of all the red cells and represents an average of 300 per cent. haemoglobin.

The strains which possessed active haemolytic properties showed very little difference in the degree of haemolysis effected in 0.5 per cent. and the 0.85 per cent. sodium chloride, or between the tubes to which red cells were added

before inoculation and those to which the cells were added the next day. With the less active strains, haemolysis was generally less marked in the tubes containing 0.5 per cent. sodium chloride.

The routine method for testing the haemolytic properties of the various strains as they were obtained was to take two, three, or more colonies from the MacConkey or blood agar plates. In the urinary cases it was found that if one colony was haemolytic the other colonies from that plate had the same property: similarly if one colony was non-haemolytic, the others were the same: on one occasion only was a haemolytic and a non-haemolytic colony obtained from the same plate: this was a case of an acute coli infection. Blood agar plates were used in the examination of faeces and when haemolysis was present, usually about one half of the colonies from that plate showed evidence of haemolysis.

Persistence of haemolytic properties. Stock cultures of all haemolytic and some non-haemolytic strains were kept and tested from time to time: the haemolytic properties were in every case retained with but slight diminution over periods varying from three months to one year. None of the non-haemolytic strains have shown any trace of haemolytic properties, while two non-haemolytic strains were subcultured ten times on blood agar, but remained non-haemolytic.

Haemolytic properties in the presence of untreated human serum and gum. Several haemolytic and non-haemolytic strains were tested with the addition of untreated human serum, and 6 per cent. gum in physiological saline, to the culture medium.

Experiment 1. In this experiment peptone water containing 0.85 per cent. sodium chloride was used and three tubes for each strain tested:

No. 1 tube contained 5 c.c. peptone water.

No. 2 " " plus 0.1 c.c. serum.

No. 3 " " " 0.05 c.c. serum.

Each tube was inoculated and incubated at 37° C. for 24 hours: 0.1 c.c. of red cells was then added and the readings taken after one hour at 37° C. Haemolysis was "complete" or "incomplete" in the case of the haemolytic strains in the No. 1 tubes, "none" in the No. 2 tubes, and "none" or "a trace" in the No. 3 tubes.

Experiment 2. To compare the action of untreated human serum and 6 per cent. gum in physiological saline (0.85 per cent.) in peptone water.

Three haemolytic strains of *B. coli* were used in this experiment and the red cells were added before inoculation of the media and the readings taken after 20 hours at 37° C. Each strain gave "complete" haemolysis in the peptone water, "incomplete" or "marked" haemolysis in the tubes to which 0.5 c.c. gum had been added, and "a trace" only in the tubes containing 0.5 c.c. of untreated human serum.

Conclusions. Numerous experiments were made by us on this question, and those quoted above have been selected as typical examples of the limitation of

haemolysis in the presence of normal serum, and little or no effect in the presence of gum: this appears to indicate that the action of the serum is something apart from mere mechanical protection, while in the case of the haemolytic streptococci the addition of blood serum induces an accentuated effect.

B. coli grown in normal untreated urine. Experiments were made with several strains of non-haemolytic urinary coli; these were inoculated in normal urine, which had been filtered through a Doulton candle: the urine was very slightly acid or neutral and contained no albumen or sugar: 5 c.c. of urine was used for each tube and these were inoculated with the strains of *B. coli* and grown for 24 hours at 37° C.

Peptone tubes with 0.5 per cent. and 0.85 per cent. sodium chloride were then inoculated from the urine cultures. There was no haemolysis in any tube whether the red cells were added at the same time or after 24 hours' growth in the peptone.

Conclusion. This experiment shows that non-haemolytic strains of *B. coli* when grown in urine do not tend to develop haemolytic properties.

The effect of growing haemolytic strains of B. coli in peptone water for some days. This experiment was undertaken to see if the haemolytic properties of *B. coli* were in any way altered by prolonged growth in peptone water without subculture.

Table I.

Shows the diminution of haemolytic properties of *B. coli* when grown in peptone water without subculture.

Organism	Degrees of haemolysis				
	I.C.	Trace	Trace	0	I.C.
4442	I.C.	Trace	Trace	0	I.C.
4879	I.C.	0	Trace	0	I.C.
Dun	I.C.	Trace	Trace	0	I.C.
Dow	I.C.	Trace	Trace	0	I.C.
X 6	I.C.	M	I.C.	Trace	I.C.
Periods of incubation at 37° C. before red cells were added	24 hrs.	3 days	7 days	11 days	24 hours' subculture from the 10 days' old culture. Then red cells added

Conclusions. The above table shows the disappearance or marked diminution of the haemolytic properties of *B. coli* when grown in salted peptone water without subculture, and the reappearance with full activity when subcultured.

SECTION II.

On *Bacillus coli* infections of the Urinary Tract.

Attention has been drawn to the fact, earlier in this communication, that some strains of *B. coli* are haemolytic while others are non-haemolytic. This difference was observed in the first instance in strains isolated by us from infected urine. Accordingly such infections were investigated more fully with the idea that the incidence of this haemolytic property would help to throw

light on the origin of these infections, about which there has been much controversy.

Method. From women the urine was collected by catheter from the bladder; in the case of men it was passed into a sterile vessel; subsequently it was centrifugalised, examined microscopically and plated direct on MacConkey plates, the amount used for plating varying with the number of bacilli seen under the microscope. Several colonies were taken, and if found to be true colon bacilli, tested for haemolysis as described in Section I. It should be mentioned that only infections caused by *B. coli* are dealt with in these observations, none are included in which "colon-like" bacilli were the infecting organisms. All strains included as colon bacilli by us produced acid and gas in lactose, dextrose, maltose and mannitol, clotted and acidified litmus milk, produced a yellow fluorescence in neutral red broth, formed indol and did not give the carbinol reaction. The fermentation of cane sugar and dulcitol will be discussed later. Inositol was not fermented by any of our strains and such as were tested did not liquefy gelatine.

In all, 69 cases of infections of the urinary tract caused by *B. coli* were examined; of these 27 were men, 42 women.

The number of haemolytic strains isolated from the two sexes is shown in the following table (II).

Table II.

Total number	Sex	Haemolytic	Non-haemolytic	Percentage of haemolytic strains	Percentage of non-haemolytic strains
27	Male	20	7	74	26
42	Female	11	31	26	74

It should be mentioned that, with one exception, all colon bacilli isolated from these infections were of one type as regards their haemolytic properties. That is, all were haemolytic or all non-haemolytic; a mixture of the two varieties was found only in the one instance mentioned above.

There is little mention in the literature of haemolytic strains of *B. coli* or of their significance.

Schottmüller and Much (1908), making a practice of plating faeces both on Conradi and blood agar from cases with any disorder of the digestive tract, found haemolytic coli from time to time. In this paper opsonic indices in various diseases are considered, and a comparison is made between the opsonic index of a patient's serum for the haemolytic and non-haemolytic colon bacillus present in the faeces in a case of gastro enteritis and jaundice.

Schmidt (1909) in a paper devoted to the significance of haemolytic *B. coli* isolated them from both urine and faeces. Most of the urines he examined were from cases of tuberculous infection of the urinary tract. He investigated the faeces also of 73 cases in which there was diarrhoea and 17 normal cases and found 72.6 per cent. haemolytic coli in cases with diarrhoea and 65 per cent. of similar organisms from the 17 normal cases. He did not attach any special

significance to the colon bacillus possessed of haemolytic properties. Haemolysis was judged by changes produced on blood agar plates.

Lyon (1917) describes a case of cystitis which he states was caused by a haemolytic *B. coli*. It was a Gram negative bacillus, but would not, on first isolation, grow on ordinary media, though it would do so later after preliminary cultivation on blood agar.

From a consideration of the previous table it appears that a haemolytic colon bacillus is by far the commonest cause of urinary infection in the case of males, whereas in women exactly the opposite occurs. This at once suggests the possibility of a different mode of infection for the two sexes. A prolonged controversy has arisen on this point in an attempt to explain the admittedly greater liability to coli infections in the female. It has been considered that in females the infection occurs as a result of the direct upward spread of bacteria over the surface of the mucous membrane of the urethra, whilst on the other hand this has been denied, and all infections of this nature have been thought to be due to a blood infection as a result of which the bacteria are excreted by the kidneys, infection extending downwards.

The upholders of the first theory point to the ease of direct contamination by faecal material in the female from anatomical considerations (Box, 1910), while those who uphold the second view consider that as intestinal stasis is more common in women, this favours a blood infection, and Kidd (1920) states that the inability to recognise the primary pyelitis followed by a cystitis later, is due to the fact that all cases have not been examined by means of the cystoscope. A large amount of experimental work has been done on this subject.

Brewer (1906) in attempting to explain acute infections of the kidney without previous cystitis showed that in animals, if the lumbar region is bruised and then living organisms are injected into a vein of the ear, an acute surgical kidney developed on the side previously subjected to trauma.

The same author also showed (1911) that injecting organisms into the circulation without previous trauma did not lead as a rule to any kidney lesion.

Lepper (1921) compressed the ureters for various periods and produced much the same results. Brewer also observed that if virulent organisms were injected into the bladder of an animal and the urethra then tied, a severe cystitis and pyelitis followed, whereas without urethral obstruction no inflammation occurred; in this last case he considered that the pyelitis thus produced was the result of an upward extension of the organism along the walls of the ureter, due to the stagnation of the urine. Thiele and Embleton (1914) showed that if bacteria are painted on the glans penis or anterior part of the urethra, care being taken to cause no abrasion, the infection spread in the following order: in one-and-a-half hours the organisms were chiefly in the iliac and renal glands; three hours later they were in the blood and chyle, while not until 15 hours later were they demonstrated in the urine, so that organisms can pass through uninjured mucous membrane up the lymphatics into the blood

stream and from there are excreted by the kidney into the urine. Further these authors showed that the same sequence of events occurred in intraperitoneal injection of living organisms, and that if the thoracic duct were opened no bacteria appeared in the urine. They could find no evidence of infection ascending along the lumen of the urethra into the bladder.

A great point has been made by those who believe that all urinary infections are primarily *via* the blood stream in that the *B. coli* can be obtained in pure culture from the blood, but the fact that such bacilli may be so recovered does not point to the origin of the infection, since in view of Thiele and Embleton's experiments, even if the colon bacillus did start from the urethral mucous membrane it would still be found in the blood.

A careful examination of the blood in *B. coli* infections of the urinary tract has shown that such bacilli may be isolated very frequently, especially if the blood is taken at the height of the rigor. Cabot and Crabtree (1916) obtained positive blood cultures in 40 per cent. of cases out of 32 examined.

In Section III it is shown that haemolytic coli frequently occur in the faeces and this fact would appear to be of considerable importance in the causation of urinary infections in man, since 76 per cent. of coli infections of the urinary tract in this sex are caused by haemolytic strains. The point therefore arises why the reverse should hold for the female? Only a small proportion of normal individuals have such haemolytic bacilli in their faeces, therefore, it would seem that in the male, and in the female, when this type of infection occurs, this bacillus is especially liable to escape from the gut into the blood stream, whether direct or *via* the lymphatics. In women the greater chance of contamination of the urethra by faecal matter must be admitted, and this being the case the greater frequency of non-haemolytic infections is explained since, as we have shown, only about 11 per cent. of normal individuals have haemolytic strains in their faeces. Whether the infection spreads upwards directly along the urethra or passes directly through the uninjured mucous membrane makes no difference to the fact that from our observations the mode of infection in the two sexes would appear to be different.

In certain instances in the male, local trauma such as a gleet or stricture may play a part in rendering possible an infection from external contamination, and this is suggested from the fact that in four out of seven of our male cases suffering from a non-haemolytic infection, there was a previous history of gonorrhoea or stricture or enlarged prostate, though the number is not great enough from which to draw any certain conclusions. Dudgeon (1908) examined 14 cases with prostatic enlargement and found *B. coli* present in five instances.

In the hope of throwing further light on this point a number of vaginal swabs were examined for the presence of the colon bacillus. They were provided by the kindness of Mr J. M. Wyatt, assistant obstetric physician to St Thomas's Hospital. Precautions were taken to avoid external contamination, the swabs when received were inoculated into a broth tube, incubated overnight, and plated on the following morning. If *B. coli* were isolated they were sub-

sequently tested for evidence of haemolysis. In all, 48 cases were examined in this manner. In 46, from the history and clinical examination, there was no reason to suppose they had a urinary infection, their complaint was, in the majority of cases, of a persistent vaginal discharge. In the remaining two cases there was a known urinary infection which had been shown to be due, in one instance, to a haemolytic *B. coli*, in the other to a non-haemolytic. In these 48 cases the *B. coli* was isolated in 13 instances. In every instance except one, the bacillus was of the non-haemolytic variety. The one exception was the case already mentioned in which the urinary infection was caused by a haemolytic strain of *B. coli*. From the remaining 11, catheter specimens of urine could be secured only in four instances; they were sterile in each case.

In four instances the husbands of women found to be suffering from a haemolytic coli infection were examined to see if they also had a urinary infection of the same type, but in each case the urine was sterile.

In some cases from which *B. coli* had been cultivated from the urine, the ureters were catheterised by Mr Cyril Nitch. *B. coli* was not always obtained from the ureteric urine, but in those instances in which positive results were obtained, the same type of organism was cultivated as in the original examination.

Haemolytic and non-haemolytic strains cultivated from the urine were tested as regards their fermentation of cane sugar and dulcitol for the presence of acid and gas as shown in the following table:

Table III.

	Males haemolytic	Males non- haemolytic	Females haemolytic	Females non- haemolytic
Cane sugar + } Dulcitol + }	2	1	1	4
Cane sugar + } Dulcitol - }	4	0	1	5
Cane sugar - } Dulcitol - }	2	0	4	10
Cane sugar - } Dulcitol + }	12	4	5	11

The organisms isolated from the vagina gave the following results:

Cane sugar + Dulcitol + 7
 Cane sugar + Dulcitol - 0
 Cane sugar - Dulcitol - 3
 Cane sugar - Dulcitol + 4

It will be seen that cultured characteristics are in no way related to haemolysis or sex, and that the greater majority of strains of *B. coli* isolated from the urine do not ferment cane sugar whether haemolytic or non-haemolytic.

Agglutination reactions. At an early stage in these observations agglutinating sera were prepared from certain urinary and faecal strains and used for testing all colon bacilli isolated from the urine. The strains used for preparing these sera were Dow and Dun, two haemolytic urinary strains, 4869

a non-haemolytic urinary strain, and X 6 and X 9, two haemolytic strains isolated from the faeces.

The results obtained offer additional evidence that infections due to haemolytic and non-haemolytic strains are of different origin. It was found that, with two exceptions, all haemolytic urinary coli were agglutinated by Dow serum. On the other hand, out of 60 non-haemolytic strains, 50 were inagglutinable with any of the anti-sera employed, while ten were agglutinated by the 4869 (non-haemolytic) anti-serum.

None of the urinary strains were agglutinated by X 6 anti-serum, whereas all the haemolytic strains (except the two already mentioned, which were inagglutinable with the Dow anti-serum) were agglutinated by the X 9 anti-serum. This is a very remarkable result and would appear to point to the fact that haemolytic colon bacilli which cause urinary infections belong to a special group of organisms which are met with in the faeces in a small proportion of normal individuals, but more frequently in cases of diarrhoea and colitis, as will be referred to in detail in a later section.

Therefore haemolytic urinary infections would seem to be the result, since they are so much more prevalent in the male where external contamination of the urethra must be uncommon, of a *primary* blood stream infection from the intestine of this special group of colon bacilli, while in the female, whether the infection is a *secondary* blood stream infection from absorption of colon bacilli from the urethral or vaginal mucous membrane, or a direct ascent of colon bacilli along the urethra into the bladder, it is the result of an external urethral contamination, in the majority of instances.

The serum Dow prepared from a rabbit had an end point of $\frac{1}{10,000}$ on its homologous antigen, as had also the sera X 6, Dun, and X 9 on their specific antigens.

As has been said all but two haemolytic strains were agglutinated by Dow and X 9 serum, though not to the end point of the serum used, but in many cases to quite a considerable degree. The degree of agglutination, however, was not in any way related to any grouping by virtue of fermentation of cane sugar or dulcitol as is shown in Table IV.

As already mentioned all organisms agglutinated by Dow serum, when tested with X 9 serum were also agglutinated, and it is shown in a later section that conversely all haemolytic faecal strains agglutinated by X 9 serum were also agglutinated by Dow serum. Agglutination of haemolytic urinary strains by X 9 serum was generally in low dilutions, though it always occurred, but one strain showed higher agglutination with X 9 serum than with Dow giving an E.P. of 1 in 3000 with the former anti-serum, but only 1 in 400 by Dow.

It has been mentioned above that two haemolytic strains which were isolated from women were not agglutinated by Dow serum. A potent anti-serum could be prepared from either by animal inoculation, but no cross agglutination between the two strains was found to occur.

Table IV.

Organism	Cultural reaction	Agglutination titre with Dow anti-serum
Parker	Dulcitol + cane sugar -	$\frac{1}{1000}$
Dun	" "	$\frac{1}{10,000}$
Weston	" "	$\frac{1}{1000}$
4702	" "	$\frac{1}{400}$
4879	Dulcitol + cane sugar +	$\frac{1}{4000}$
Peters	Dulcitol - cane sugar -	$\frac{1}{1000}$
4864	" "	$\frac{1}{400}$
Haines	" "	$\frac{1}{1000}$
4442	Dulcitol - cane sugar +	$\frac{1}{400}$
5229	" "	$\frac{1}{1000}$

SECTION III.

On the Examination of Faeces for the presence of
Haemolytic *Bacillus coli*.

It appears probable that in some cases of bacilluria and pyuria caused by a haemolytic strain of *B. coli*, the infecting organism may gain entrance to the urinary tract *via* the blood stream. If so such organisms may have their normal habitat in the intestinal canal; accordingly the faeces of various classes of patients were examined for the presence of haemolytic colon bacilli.

Method. The faeces were dried on a tile by the method advocated by Dudgeon, and a quantity of the dried powder was evenly spread over a blood agar plate, Wordley (1921), made by adding 1 c.c. of undiluted human oxalated blood to 15 c.c. of melted agar. The plates were incubated over-night and examined next morning for the presence of haemolytic colonies. Haemolytic coli colonies¹ show a narrow, faint, rather irregular zone of haemolysis, quite unlike the well-defined zone round a haemolytic streptococcus colony, and as a rule this can be seen best by looking through the plate. Haemolytic colonies when present were subcultured on to an agar slope and their haemolytic properties were further determined by the methods already described, while the cultural and serological characters were determined also. When haemolytic coli colonies were present on blood agar plates they were found generally in large numbers, at least a half being of this nature. In many instances all coliform

¹ The only evidence of haemolysis may be a greenish discoloration of the colonies.

colonies present showed evidence of haemolysis. While some colonies showing evidence of haemolysis on a blood agar plate did not always do so when inoculated in a peptone water red cell mixture, the reverse of this was not observed. All non-haemolytic colonies on a blood agar plate were found to be of the same nature when tested for haemolysis by other methods.

The cases which furnished material for these investigations are grouped as follows:

I. "*Enterica*" and *Dysentery*. Fifteen patients were included in this group, who, on bacteriological or microscopical evidence, had been infected within three months of the present investigations.

II. Twenty cases were examined. These patients had suffered from diarrhoea and had passed blood or mucus or both, but on repeated examinations of the stools, bacilli of the "*enterica*" or dysentery group were not isolated, and the *entamoeba histolytica* was not found.

III. Eleven cases of diarrhoea in infants under two years of age were examined.

IV. Fourteen cases of acute and chronic nephritis.

V. Thirty-three cases are referred to as "unclassified." These include various diseases not especially associated with any obvious intestinal lesion.

VI. Three cases of pernicious anaemia.

VII. Thirty-nine cases labelled as "normal." These patients were admitted to hospital for operation for hernia, deflected nasal septum or for enlarged tonsils.

The final results can best be appreciated if expressed in tabular form.

Table V.

Group	No. of patients examined	Nature of case	Positive results
I	15	"Enterica" and dysentery	5
II	20	Colitis	7
III	11	Diarrhoea in infants	4
IV	14	Nephritis	3
V	33	"Unclassified"	7
VI	3	Pernicious anaemia	1
VII	39	"Normal cases"	5

It will be seen that of these 135 patients, 32 or 24 per cent. had haemolytic strains of *B. coli* in their faeces.

In 39 "normal" cases there were five cases or 13 per cent. from which haemolytic coli were cultivated, while in 31 cases of diarrhoea or colitis, 11 or 35.4 per cent. showed haemolytic coli. The presence of haemolytic coli in the faeces bore no relation to sex.

The cultural characteristics of the haemolytic strains of organisms were fully investigated but no great advantage would be gained by a detailed description. The greater number fermented both dulcitol and cane sugar as can be seen from the following table.

Table VI.

		No.
1	{ Cane sugar } + { Dulcitol }	16
2	{ Cane sugar - } { Dulcitol + }	5
3	{ Cane sugar + } { Dulcitol - }	5
4	{ Cane sugar } - { Dulcitol }	3

Dr E. W. Todd working in this laboratory permits us to quote from some observations of his, as yet unpublished. Investigating the faeces of infants under one year old not suffering from diarrhoea he found, in 100 cases examined, 13 instances in which haemolytic strains of *B. coli* were present, a proportion identical with that obtained by us in normal adult cases. We examined eight of these 13 strains to determine their serological relationship with the various anti-sera we have prepared. Of these eight, six were agglutinated by X 9 and Dow anti-sera and not by X 6 serum, while two were inagglutinable by any of our anti-sera.

Immune sera for agglutination tests were prepared from rabbits with two of these haemolytic coli (X 6 and X 9) and were employed to test the remainder with the following results. In addition the majority were tested serologically with anti-sera prepared from Dow a haemolytic urinary strain, and "Hyde," a haemolytic culture isolated from the blood.

Cultural characteristics as regards the fermentation of dulcitol and cane sugar bore no relation to agglutination, different types culturally being agglutinated equally well by the same serum.

It was found that eight organisms were agglutinated by X 6 serum and by no other serum. Four strains were agglutinated by X 9 serum and further these four were all agglutinated by Dow serum. None were agglutinated by "Hyde" serum.

Our observations show that haemolytic strains of *B. coli* were most prevalent in cases of diarrhoea or colitis. The initial object of these investigations was to ascertain whether haemolytic coli isolated from the faeces were serologically identical with the Dow urinary strain, since the vast majority of haemolytic urinary strains are agglutinated by an anti-serum prepared from this bacillus. If it was found to be of common occurrence for intestinal coli to be agglutinated by the Dow anti-serum it would help to throw light on the origin of coli urinary infections. Amongst the strains we have examined this phenomenon was not found to be of common occurrence; on the other hand it has been found that a distinct serological relationship exists between X 9 and the haemolytic urinary organism Dow, in that all faecal strains agglutinated by X 9 were also agglutinated by Dow serum. Conversely all haemolytic urinary strains, except the two inagglutinable already mentioned, were also agglutinated by X 9 and not by X 6 serum.

Treatment by vaccines. Three cases, in the faeces of whom large numbers of haemolytic coli were present, were treated with vaccines. In each case their haemolytic organism was agglutinated by X 6 serum. Accordingly, a vaccine was prepared from this organism. These three patients were suffering from chronic colitis, colitis following removal of the colon for dysenteric ulceration, and coeliac disease, the latter being an infant under two years of age. In every case very marked improvement followed. The disease in each case was of long duration and a variety of treatments had been tried previously.

It is interesting to note that in one of the above cases the faeces were again examined after the colitis had subsided when no haemolytic coli were found.

Examination of human appendices for the presence of haemolytic strains of B. coli. While the above investigations were in progress the opportunity was taken to see if haemolytic strains of *B. coli* were of common occurrence in the appendix in appendicitis.

The appendix removed at operation was incised and a quantity of material from the lumen inoculated on to a MacConkey plate. From the resulting growth four colonies were taken and tested for evidence of haemolysis, in no instance was haemolysis observed. In all 20 appendices were examined in this manner, of which seven were necrotic, eight were acutely inflamed, and five showed no naked eye evidence of inflammation.

Examination of water contaminated by sewage for haemolytic colon bacilli. Through the kindness of Sir Alexander Houston, K.B.E., a number of cultures of the colon bacillus obtained from river water at its raw source of supply were examined for haemolytic properties. In all 22 strains were examined. In no case did these organisms have haemolytic properties.

SECTION IV.

Examination of the sera of normal individuals and others was made for the presence of *B. coli* agglutinins.

(a) The serum of "normal" individuals, by which is meant those who there was no reason to suppose were suffering from a *B. coli* infection, was tested with our standard antigens. While we fully realise that this is an extremely difficult question to decide, yet a similar objection applies to the definition "normal" throughout medical practice. The normal sera were mostly obtained from patients whose blood was being examined for the Wassermann or some other reaction, and such samples of sera were tested with our *B. coli* antigens, more especially "Dow" the haemolytic urinary strain. Up to the present only a very limited number of such examinations have been made—66 in all; but of this number 61 were negative in a dilution of 1 in 50 to these antigens with a 1 in 10,000 end-point, and to the non-haemolytic antigen which acted with its immune serum to 1 in 800. In five instances a positive reaction occurred.

The results are expressed as follows:

Table VII. Normal sera.

No. examined	Negative	Positive	
	Dil. 1 in 50	1 in 50	1 in 400
66	61	2	3

Of the positive cases (5) the urine was examined in two and found to be sterile.

(b) Sera from patients with infection of the urinary tract due to *B. coli*.

Fourteen such cases were examined of which six showed a reaction varying from 1 in 50 to 1 in 400.

Table VII a.

No. examined	Negative	Type of infection		Positive. Antigens				
				Haemolytic			Non-haemolytic	
				1 in 400	1 in 100	1 in 50	1 in 100	1 in 50
14	8	Haemolytic	5	4*	1	—	1*	—
		Non-haemolytic	1	—	—	1	—	—

It will be seen in the above table that the serum of one case (*) due to a haemolytic strain of *B. coli* agglutinated the haemolytic standard antigen as well as the non-haemolytic. This patient had had "cystitis" for 15 years.

(c) Ten cases were examined which were grouped as intestinal, and in four instances the serum of these patients agglutinated haemolytic coli antigens in dilutions varying from 1 in 50 to 1 in 200.

(d) Five cases, any of which may have had a *B. coli* infection, although it was not proved, reacted with one of the haemolytic antigens in dilutions varying from 1 in 100 to 400. In one case, the reaction on the first occasion was negative, but 14 days later, as the pyrexia continued, the blood was re-tested and the reaction was then 1 in 400.

(e) The blood of ten cases which had received a course of *B. coli* vaccines in doses from 50–1000 million were re-examined, but in only three instances a definite rise in the agglutinin content of the serum had occurred.

In all these observations the standard antigens employed by us were a haemolytic urinary antigen "Dow," two haemolytic faecal strains X 6 and X 9 and one non-haemolytic urinary antigen 4869.

SECTION V.

On the Immunisation of Rabbits.

When it was found that *B. coli* isolated from the urine could be separated into two groups (1) haemolytic; (2) non-haemolytic, experiments were commenced for the preparation of immune anti-sera.

At first rabbits were injected I.V. with formol-killed vaccines, but it was evident at an early date that immunisation with our non-haemolytic strains

was abnormally difficult to effect. For this reason it is necessary to refer concisely, but briefly, to the methods which we adopted in our immunisation experiments.

Methods: (a) Vaccines.

(1) *Formol-killed.* Young agar cultures (24 hours) were emulsified in normal saline (0.85 per cent.) containing 0.1 to 0.14 per cent. formaldehyde, filtered through sterile linen, and stored in the ice safe until they were dead, when suitable dilutions were made with saline containing 0.25 per cent. phenol.

(2) *Heat-killed.* Young agar cultures were employed also for these experiments, but attempts were made to reduce the temperature of the water and the time of exposure to the lowest and shortest possible. Numerous experiments were made on these lines (Table VIII), but we failed to satisfy ourselves that the minimum period and lowest temperature produced any special advantages over the time and temperature limit which we employ as a routine procedure.

Thermal death point. Cultures of *B. coli* on agar were 24 hours old. Saline emulsions were made which were put in the water bath for the required period, incubated at 37° C. for 24 hours, and subcultured on agar. These subcultures were left for varying periods at 37° C. as stated below. The amount of vaccine in each tube was about 3 c.c. and the diameter of the test tubes employed was $\frac{5}{8}$ inch.

Table VIII.

Haemolytic strains isolated from the urinary tract.

Name or No. of strain	Strength	Temp. of water bath	Time of exposure	Result
Gooch	1000 million	59° C.	40 mins.	Sterile in 5 days
Dun	{1000 5000}	"	"	Sterile in 48 hours
4442	{1000 5000}	"	"	Sterile in 72 hours
Peters	{5000 1000}	"	"	"
Dow	{1000 5000}	"	"	"
Dun	{2000 2000}	"	{30 mins. 15 mins.}	"

Non-haemolytic strains of *B. coli* isolated from the urinary tract gave similar results. At the lower temperatures, such as 57° C. or 55° C., very variable results were obtained, whilst at 59° C. if the period of exposure was reduced to 15, 20 or 30 minutes equally variable results occurred.

(b) *Live cultures.* Young agar cultures emulsified in sterile saline, and stored in the ice safe during the course of our experiments, which varied from one to two months, were also employed.

(c) *Animal inoculation.* The rabbits were injected intravenously, intramuscularly or subcutaneously, and to a much less extent intraperitoneally.

Lepper (1921) made certain observations on the production of agglutinins in rabbits after the injection of various strains of *B. coli* and found that high titre agglutinins are produced only for the homologous strain, while group agglutination based on the fermentation of cane sugar and dulcitol does not occur, although members of the same group would agglutinate to a limited degree.

Herrold and Culver (1919) investigated 43 cases of "renal infections" caused by *B. coli*. They prepared four immune sera from strains separated by cane sugar—dulcitol reactions (CS + D +; CS - D -; CS + D -; CS - D +). The end point of each serum was 1 in 1280 for the autogenous strain, while the highest titres obtained when cross agglutination was attempted were 1 in 640 and 1 in 320. Apart from these two results no agglutination was observed between various strains of the same group or any other group.

On the results of intravenous inoculation. Our investigations were undertaken for the purpose of studying some of the many problems associated with the bacteriology and pathology of coli infections of the urinary tract, but we found it necessary to extend our observations to the intestinal tract. Bacteriological investigations of the faeces resulted in our finding haemolytic strains of *B. coli* under varying conditions. It was thus obvious that attempts to immunise animals with haemolytic strains (group 1) and non-haemolytic (group 2) isolated from the urine, and haemolytic strains obtained from the faeces (group 3), were essential, so as to compare if any serological affinity, based on agglutination records, existed and whether "grouping" could be attempted.

Rabbits were first immunised I.V. with formol-killed vaccines of the haemolytic urinary coli (Dow). This was readily accomplished as six injections of 100–1000 million bacilli at weekly intervals resulted in an anti-serum with an end point of 1 in 10,000 to the specific bacillus; in each instance rabbits rapidly responded to I.V. injections with this organism. The anti-serum (Dow) has proved of the utmost value in grouping colon bacilli isolated from the urine, and, as shown elsewhere, in agglutinating some strains of haemolytic coli isolated from the faeces.

Other rabbits were inoculated with other haemolytic strains of coli obtained from the urine, faeces and blood stream; the injections were made with vaccines and live cultures.

Injections of 10,000 million were employed in some instances for the immunisation, as massive doses produced no ill effects, but no special advantage was obtained. A high titred serum was produced in every instance by injecting rabbits with a haemolytic colon bacillus I.V. In Table IX are included various data which illustrate the results of the immunisation of rabbits with two of these haemolytic coli (groups 1 and 3), isolated from urine and faeces. The agglutinins formed in each rabbit were found to be strictly specific; the animals were never ill during any stage of the experiments, although the injections were made with live bacilli.

Table IX.
Rabbits (10 and 11).

10. Haemolytic *B. coli* from urine (Dow).
11. ,, ,, faeces (X 6).

Immunisation methods. These rabbits were injected I.V. with the live bacilli. Rabbit 11 received *B. coli* (Dow), and rabbit 10 *B. coli* (X 6). The inoculations were carried out in an identical manner and the agglutinin content of the serum of each rabbit is recorded for its specific bacillus.

Date	Number of bacteria injected	Agglutination titre. <i>B. coli</i> antigens	
		X 6	Dow
11. x. 20	50 million I.V.	0	0
15. x. 20	100 ,,	50	50
		10,000	10,000
		200	400
22. x. 20	200 ,,	10,000	10,000
		5000	5000
		10,000	10,000
29. x. 20	500 ,,	10,000	10,000
		10,000	10,000
		10,000	10,000
3. xi. 20	-	10,000	10,000

Though the results referred to were obtained without difficulty when the haemolytic strains were employed, yet with the non-haemolytic coli isolated by us from the urine only relatively feeble immunisation was effected, whether vaccines heat or formol-killed, or live cultures were employed¹. Numerous strains were tried with very similar results—in fact, there was only one non-haemolytic culture of *B. coli* (4869) isolated from the urine which excited the formation of agglutinins in the rabbit, and then but relatively feebly. The end point of the various anti-sera obtained with this organism failed to exceed 1 800 with the appropriate antigen². Various methods were employed, but whether doses of 100 or 10,000 million bacilli were injected no greater response was effected, and I.V. injections of *Staphylococcus aureus*, typhoid, paratyphoid, or haemolytic coli vaccines were ineffective.

This result is at variance with that obtained by Dreyer and Ainley Walker (1910) with *B. coli*, who found that “the injection of various heterologous bacteria increases the production of special agglutinins in an immunised animal so long as it retains any measurable degree of acquired immunity.” In Table X is illustrated the effect of I.V. injection of a haemolytic and two non-haemolytic urinary strains of *B. coli*. The rapid immunisation to the haemolytic strain, and the absence of response with one of the non-haemolytic and the comparatively feeble response with the other is fully emphasised, while the failure of a typhoid and other vaccines employed to produce the formation of agglutinins for the non-haemolytic strain is fully illustrated. •

¹ The sera of the inoculated rabbits produced clumping of the appropriate antigens, but only in low dilutions and with unsatisfactory clumps.

² We subsequently, after many trials, have obtained two active immune sera in rabbits.

Table X.

- Rabbit 2. (1) Non-haemolytic *B. coli* from urine (4657).
 (2) *B. typhosus*.
 (3) Haemolytic *B. coli* (Dow).
 (4) Non-haemolytic *B. coli* (4869).

Immunisation methods. This rabbit received I.V. three strains of *B. coli*, as stated above, which were isolated from the urine, and a vaccine of *B. typhosus*. Three of the vaccines were formol killed, while the non-haemolytic strain (4869) of *B. coli* was injected I.V. as the living organism.

Date	Number of bacteria injected		Agglutination titre			
			<i>B. coli</i>			<i>B. typhosus</i>
			Dow	4657	4869	
15. iv. 20	100 million	4657	—	—	—	—
19. iv. 20	500	„	—	0	—	—
23. iv. 20	500	„	—	0	—	—
3. v. 20	1000	„	—	0	—	—
11. v. 20	1000	„	—	0	—	—
18. v. 20	200	<i>B. typhosus</i>	—	0	—	0
27. v. 20	500	„	—	0	—	200 8000
28. v. 20	1500	4657	—	—	—	—
1. vi. 20	100	<i>B. Dow</i>	0	0	—	1000 8000
7. vi. 20	250	„	100 10,000	0	—	1000 8000
14. vi. 20	500	„	1000 10,000	0	—	—
21. vi. 20	1000	„	2000 10,000	0	—	—
28. vi. 20	1250	„	7500 10,000	0	—	—
5. vii. 20	2000	„	—	—	—	—
16. vii. 20	50	<i>Live 4869</i>	—	—	0	—
23. vii. 20	200	„	—	—	—	—
6. viii. 20	400	„	10,000 10,000	0	1 in 100	—
12. viii. 20	400	„	—	—	1 in 200	—
19. viii. 20	800	„	—	—	—	—
25. ix. 20	2000	„	10,000 10,000	0	1 in 400	—
11. x. 20	—	„	10,000 10,000	0	1 in 800	—

Intramuscular and subcutaneous inoculation. It has been said already that the immunisation of rabbits I.V. with haemolytic urinary and intestinal colon bacilli, and the feeble effect provoked with the non-haemolytic strains, were constant. By the above methods of inoculation, whether live, heat and formol killed vaccines are employed, and whatever dosage is administered, non-haemolytic strains are similarly inactive, while the haemolytic strain may not

be nearly so effective. It is therefore unnecessary to refer in detail to the rabbit experiments with non-haemolytic strains and confine our remarks to the haemolytic cultures of *B. coli*.

The result of very numerous experiments with these haemolytic urinary and faecal strains of *B. coli* (whether vaccines or live cultures, employed in doses varying from 100–2000 million bacilli) has demonstrated that the subcutaneous route for immunisation of animals cannot be relied upon as far as production of agglutinins in the blood of rabbits is the main object. An anti-serum with a titre of $\frac{5000}{10,000}$ may be produced, but in many instances five and six inoculations with doses varying from 100–2000 million bacilli resulted in a serum with an end point of $\frac{400}{10,000}$. If, however, a typhoid vaccine was injected subcutaneously rapid immunisation occurred, or if a haemolytic colon bacillus was subsequently inoculated I.V. a similar active formation of agglutinins occurred, but without effect on the agglutinin content of the serum for the bacillus injected subcutaneously.

In Table XI are illustrated the varying results which followed when subcutaneous immunisation of rabbits was employed with heat killed vaccines followed by live cultures.

Table XI.

	A	B	C
Organism employed	Haemolytic urinary coli (Dow)	Two strains from faeces. X 6; X 9	Two haemolytic urinary coli cultures. (1) Dun; (2) Smith
Duration of experiment	6 weeks	3 months	5 weeks
No. of injections	7	14	8
No. of bacteria injected	5200 million	{ 7700 million X 6 4100 „ X 9	5100 million Dun 1600 „ Smith
Titre of serum	5000 Dow	200 X 6	200 Dun
	10,000	10,000	10,000
		400 X 9 10,000	50 Smith 10,000

On the inoculation and feeding of rabbits with live cultures of B. coli. Lepper injected rabbits with living cultures of *B. coli* and produced a transitory bacteraemia with large doses (4000 mills.). Of these rabbits one was killed within 24 hours as it was very ill; inoculated in this manner, the *B. coli* was recovered from 0.01 c.c. of blood at the autopsy. The other two gave positive blood cultures at end of second day, but the blood was sterile by the fourth day.

Doses of 1000 million bacilli were injected into five rabbits, but a proved blood infection was produced in one instance only, while in a further series of experiments with 15 rabbits, kidney lesions due to coliform infections occurred in three.

Many rabbits were injected by us with live cultures of haemolytic urinary and faecal coli and non-haemolytic urinary coli, but without effect, apart from

a local abscess which formed in a few instances at the seat of injection. Two experiments are referred to in detail to emphasise the fact.

Experiments X. Rabbit 5 had just previously received seven injections of a vaccine of a non-haemolytic strain of *B. coli* (4657), isolated from the urine. The injections had been administered I.V. and the last injection was a fortnight before the present experiments commenced. This rabbit had failed to form agglutinins. It now received I.V. injections of the living bacillus (4657) in doses of 50–200 and 400 millions at weekly intervals. One week after the last injection it was killed under chloroform. A complete post-mortem examination was made immediately after death. There was nothing abnormal to the naked eye.

Cultures were made from the heart blood, the urine, muscles, spleen, kidneys and from the bile. All cultures remained sterile. The urine was clear and showed no deposit.

Experiments Y. Rabbit 17 had received during a period of two months six injections I.V. of a living culture of a haemolytic coli isolated from the urinary tract (Smith) and two inoculations subcutaneously of the same organism.

The animal was killed one week after receiving 500 million of the live colon bacillus subcutaneously.

Post-mortem examination was made immediately after death (chloroform anaesthesia). There was slight redness of the tissues at the seat of the last injection of 500 million of living coli one week previously, but no other changes were observed.

Cultures were made from the heart blood, muscle at seat of redness, urine, bile, liver, spleen and kidneys.

All cultures remained sterile.

On the feeding of rabbits. Three rabbits were used for feeding experiments with live cultures of *B. coli* (X 6). Rabbit 1, which had been used for immunisation, with a living culture of X 6, was fed with same haemolytic faecal strain (X 6). The second had been injected in a similar manner with Dow. The third was a normal healthy rabbit on which no experiments had been performed.

The method employed was to saturate sliced up carrots with a thick emulsion of the haemolytic faecal colon bacillus (X 6). A daily feed was given to these three rabbits over a period of 18 days, but in each case the rabbits remained in perfect health. They were killed at the end of the period mentioned, but no lesions were apparent on naked eye examination of any of the viscera at the autopsy.

During the last ten days of these experiments in addition to the feed of bacilli and carrots, 1 c.c. of a 2 per cent. solution of bile salt with 0.5 c.c. of a 10 per cent. solution of calcium chloride was added, with the idea that some irritation of the bowel might be produced and thus enhance any effect of the live culture. This procedure made no difference to the health of the rabbits, who showed no signs of any intestinal disturbance and, as has been said, remained in perfect health throughout. At the post-mortem examinations

cultures were made from the heart's blood, liver, kidney, muscle, bile, and urine from all three rabbits, but in every case the cultures were sterile.

During the course of these experiments the animals were bled every six days, and their sera tested on an X 6 antigen for evidence of agglutination. In one case the serum of the control rabbit showed that agglutinins had been formed as the result of these feeding experiments.

Table XII.

End point agglutination of the rabbits' sera with a standard antigen X 6.

Rabbit	Before feeding	After feeding
X 6 live	2000	2000
	10,000	10,000
Dow live	0	0
	10,000	10,000
Control	0	400
	10,000	10,000

The conclusions derived from these experiments on the immunisation of rabbits with haemolytic coli cultures isolated by us from the urine, faeces and blood, and with non-haemolytic coli from the urine are as follows:

(1) There was considerable difference in the immunising effect induced by haemolytic and non-haemolytic strains of *B. coli*. All our haemolytic strains when injected I.V., isolated from urine and faeces, excited an active formation of agglutinins in the blood of rabbits, while with non-haemolytic urinary strains this did not occur.

(2) Only one non-haemolytic urinary strain provoked agglutinin formation in the rabbit and then relatively feebly as compared with the haemolytic¹.

(3) Agglutinins were readily formed in the blood of rabbits with the haemolytic strains when the injections were made I.V. with heat or formol killed vaccines, or live cultures.

(4) If the injections were made subcutaneously with living haemolytic cultures, or vaccines, agglutinins would be formed, but as a rule much less effectively than by the I.V. route.

(5) No advantage was gained in the immunisation of rabbits with these various strains of haemolytic coli if a minimum time period and a low temperature were employed in the preparation of the heat-killed vaccines.

(6) Injection of typhoid, paratyphoid, haemolytic coli, and staphylococcal vaccines had no effect on the production of non-haemolytic coli agglutinins, if injected with the specific vaccines.

(7) The injection of live coli cultures into rabbits intravenously, intramuscularly or subcutaneously, was not associated with harmful effects apart from abscess formation at the site of injection.

(8) Our feeding experiments with living cultures of a haemolytic *B. coli* isolated from the faeces failed to produce any effect, on clinical or pathological evidence, on the rabbits, except in the formation of agglutinins referred to above.

¹ See footnote 2, page 153.

SECTION VI.

Bacillus coli Agglutinins.

1. *Methods employed.* Numerous methods were studied for determining the presence of coli agglutinins in a sample of serum, for estimating the titre, and for ascertaining the agglutinability of various strains of urinary and haemolytic faecal coli. Many of the details employed by us are based on the work of Dreyer and his colleagues on typhoid and dysentery agglutinins. We found, after extensive trials, that the most satisfactory temperature for the reaction is 50–55°C. for a period of five hours; the reaction should be read after the Dreyer tubes have stood for one hour at room temperature. Coli “clumps” are small and show less tendency to fall than the typhoid, while there is a strong tendency for a “trace of agglutination” to occur over a wide limit of dilutions. The sharp gradation which is so characteristic in the typhoid-paratyphoid reaction is uncommon. “Zonular agglutination” is not infrequently met with. Strains of *B. coli* isolated by us were tested with the sample of anti-sera in a somewhat similar manner to the true Weil-Felix reaction. Young agar cultures were employed, the growth was emulsified in 2 c.c. of normal saline and then one drop of the thick emulsion was added to tubes containing 1 c.c. of diluted anti-serum; the whole was incubated in a water bath for five hours at 50–55° C. as already stated and the results duly recorded.

2. *Preparation of antigens.* Standardised antigens were employed with some of our haemolytic urinary strains, of which “Dow” and “Dun” were most frequently made use of, a haemolytic strain from the blood “Hyde,” and haemolytic strains from the faeces X 6 and X 9, while a non-haemolytic urinary strain most commonly employed was No. 4869. The bacilli were subcultured daily for at least ten days on agar, then planted on agar in Roux flasks or flat whisky bottles, so as to obtain a massive surface growth which was emulsified in normal saline containing 0·1–0·12 per cent. formaldehyde, and stored in the ice safe until proved to be sterile. Dilution of the antigen was made with normal saline (0·85 per cent.) containing 0·25 per cent. phenol, until a strength of 1000 million bacilli per c.c. was obtained. Antigens prepared in this way were generally found to be very satisfactory, but occasionally they had to be discarded as the sensitiveness was insufficient. Thin antigens, owing to the very fine clumps formed, were found unsuitable, but antigens of 1000 millions proved most satisfactory.

We had made various trials with beef broth antigens, but the results were generally less satisfactory than the agar antigens. Still we found from experience that it was necessary when testing strains of *B. coli* grown on agar which were inagglutinable with stock anti-sera, to re-examine the organisms grown in beef broth, and one of our standard antigens was made in beef broth in preference to agar. Experiments with veal broth antigens are in progress.

The antigens were tested with our stock anti-sera when first made, and at subsequent intervals, so as to correct any alterations that might occur, but after a period of six weeks from the time of the preparation a constant "end point" was obtained, as freshly made antigens were generally found to be most sensitive and then to fall to a constant level.

Gardner (1918) had shown from elaborate experiments with cultures of *B. dysenteriae* that a period of two months must be allowed to elapse before the emulsions can be used as standard, because of the action of the formalin.

The readings were taken in the dark against a black background and mostly by one of us (L. S. D.) with the aid of a hand lens which was found to be essential. The results were expressed as (i) "Complete" (clear tube with solid deposit of bacteria); (ii) "Incomplete" (few clumps suspended); (iii) "Marked" (numerous suspended clumps and a deposit); and (iv) "Trace" - end point (no deposit; fine clumps suspended in the fluid) is recorded by giving the end point either of the serum or antigen tested with standard antigen or stock anti-serum over the end point of the stock anti-serum and standard antigen as follows:

$$(1) \quad \frac{600}{10,000} = \frac{\text{"Test serum" + standard antigen}}{\text{Stock anti-serum + standard antigen}}$$

or

$$(2) \quad \frac{800}{10,000} = \frac{\text{"Test antigen" + stock anti-serum}}{\text{Standard antigen + stock anti-serum}}$$

This is the same method as employed by one of us (Dudgeon, 1919) for the records on dysentery agglutinins.

3. *Saturation experiments.* The saturation experiments were carried out on similar lines to those employed by one of us (Dudgeon, 1919) for dysentery bacilli. Massive growths from agar cultures 24 hours old were emulsified in a minimum quantity of normal saline. The "saturation" was attempted by the fractional method; to a measured amount of anti-serum an equal quantity of bacillary suspension was added in fractions $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{4}$ during the 20-24 hours the experiment was in progress, as shown in Table XIII.

Table XIII.

Anti-serum	Bacillary suspension	Time	Temperature
1 c.c.	0.5 c.c.	11 a.m.	52° C.
—	0.25 c.c.	2 p.m.	52° C.
—	0.25 c.c.	4 p.m.	52° C.
—	—	7 p.m.	Ice
		11 a.m.	(Experiment completed)

The final mixture was then centrifuged at high speed until "cleared from bacilli," when it was ready for testing. Some experiments, however, all but the first stage which took place at 50-55° C. were completed in the ice safe. No obvious difference resulted from this technical alteration.

Experiment 1. Anti-serum Dow (haemolytic urinary type).

This serum was tested on the following strains of *B. coli* with the results recorded in Table XIV.

Table XIV.

Organism	Source	Type	Agglutination titre (anti-serum Dow)
1. Dow	Urine	Haemolytic	1 in 10,000
2. Dun	„	„	1 in 8000
3. 4657	„	Non-haemolytic	0
4. 4869	„	„	0
5. X 6	Faeces	Haemolytic	0
6. X 9	„	„	1 in 400
7. 4442	Urine	„	1 in 400
8. 4879	„	„	1 in 400

The anti-serum Dow was then saturated with the same eight strains of *B. coli* referred to above with the following results recorded in Table XV.

Table XV.

Organism	Strength of emulsion	Treated Dow serum on Dow antigen
1. Dow	80,000 million per c.c	400 10,000
2. Dun	40,000 „	400 10,000
3. 4657	40,000 „	10,000 10,000
4. 4869	50,000 „	10,000 10,000
5. X 6	80,000 „	10,000 10,000
6. X 9	70,000 „	1000 10,000
7. 4442	40,000 „	5000 10,000
8. 4879	40,000 „	5000 10,000

Experiment 2. Anti-serum 4869 (non-haemolytic urinary type).

This serum was tested on the following strains of *B. coli* with the results recorded in Table XVI.

Table XVI.

Organism	Source	Type	Agglutination titre (anti-serum 4869)
1. 4869	Urine	Non-haemolytic	1 in 800
2. Dow	„	Haemolytic	0
3. X 6	Faeces	„	0

The anti-serum was then saturated with the same three strains of *B. coli* with the following results recorded in Table XVII.

Table XVII.

Organism	Strength of emulsion	Treated 4869 serum on 4869 antigen
1. 4869	50,000 million per c.c.	50 800
2. Dow	80,000 „	800 800
3. X 6	80,000 „	800 800

Experiment 3. Anti-serum X 9 (haemolytic faecal type).

This serum was tested on the following strains of *B. coli*, and the results are recorded in Table XVIII.

Table XVIII.

Organism	Source	Type	Agglutination titre (anti-serum X 9)
1. X 9	Faeces	Haemolytic	1 in 10,000
2. Dow	Urine	„	1 in 800
3. X 6	Faeces	„	0
4. 4442	Urine	„	1 in 1000
5. 4864	„	„	1 in 1000
6. 4869	„	Non-haemolytic	0

The anti-serum X 9 was then saturated with the same six strains of *B. coli* referred to in the above table with the results recorded in Table XIX.

Table XIX.

Organism	Strength of emulsion	Treated X 9 serum on X 9 antigen
1. X 9	70,000 million per c.c.	50 10,000
2. Dow	70,000 „	1000 10,000
3. X 6	65,000 „	8000 10,000
4. 4442	90,000 „	1000 10,000
5. 4864	70,000 „	1000 10,000
6. 4869	50,000 „	10,000 10,000

Experiment 4. Anti-serum Smith (haemolytic urinary strain).

This serum was prepared from a haemolytic urinary strain of *B. coli* (Smith) which did not agglutinate with any of our coli anti-sera. The following strains of *B. coli* were tested with this anti-serum with the results recorded below.

Table XX.

Organism	Source	Type	Agglutination titre (anti-serum Smith)
1. Smith	Urine	Haemolytic	1 in 10,000
2. X 9	Faeces	„	0
3. X 6	„	„	0
4. Dow	Urine	„	0
5. 4869	„	Non-haemolytic	0

The anti-serum Smith was then saturated with the same five strains of *B. coli* referred to above with the following results recorded in Table XXI.

Table XXI.

Organism	Strength of emulsion	Treated Smith serum on Smith antigen
1. Smith	50,000 million per c.c.	50 10,000
2. X 9	70,000 "	} 8000 10,000
3. X 6	80,000 "	
4. Dow	80,000 "	
5. 4869	50,000 "	10,000 10,000

The experiments illustrated in Tables XV, XVII, XIX, XXI were selected because they serve to show the effect of "saturating" four types of anti-coli serum: (i) common haemolytic urinary "Dow"; (ii) non-haemolytic urinary "4869"; (iii) haemolytic faecal "X 9" which has *definite* serological affinity with "Dow"; (iv) haemolytic urinary (Smith) which is up to date a solitary strain of haemolytic *B. coli* isolated from urine without serological affinity for any other colon bacillus which we have investigated so far. These tables simply serve to show some of the results which we have obtained in this early stage of our investigation, but in some instances it was found that considerable de-saturation of an anti-serum was effected by a coli strain which had little "affinity" as judged by the agglutination results. Our research on this difficult question is not sufficiently advanced to permit us to enter into the numerous complications surrounding this branch of the subject.

The main conclusions which are warranted from our saturation results up to date are as follows:

(1) The common type of haemolytic urinary colon bacillus which is represented by "Dow" furnishes an anti-serum which is partially or completely de-saturated by the majority of other haemolytic urinary strains.

(2) Non-haemolytic urinary strains do not, as far as our investigations permit us to affirm, de-saturate an anti-coli serum prepared from a haemolytic urinary colon bacillus. The converse is equally true.

(3) We have cultivated haemolytic colon bacilli from the faeces which have definite serological affinity for the standard haemolytic urinary coli anti-serum "Dow" as shown by agglutination and saturation experiments.

CONCLUSIONS.

(1) *Bacillus coli* in infected urine can be divided into two groups: (i) haemolytic; (ii) non-haemolytic.

(2) The haemolytic group is the common type in the infection in men and the non-haemolytic in women.

(3) Rabbits can be readily immunised with the haemolytic strains, but this is not so with the non-haemolytic which we investigated.

(4) Haemolytic *B. coli* occur in the normal faeces of adults and infants, but with increased frequency in cases of diarrhoea and colitis.

(5) A serological relationship exists between some haemolytic urinary and faecal strains of *B. coli*.

(6) All haemolytic urinary strains of *B. coli*, with two exceptions, were agglutinated by an anti-serum prepared from one strain.

(7) Non-haemolytic strains are not agglutinated by one anti-serum as is the case with the haemolytic.

(8) I.V. inoculation in animals with haemolytic strains of *B. coli* leads to the rapid formation of agglutinins; when vaccines or live cultures are injected subcutaneously or intramuscularly in man and animals, feeble response may occur.

(9) The only harmful effect of injecting intravenously, intramuscularly or subcutaneously live cultures of haemolytic or non-haemolytic strains of urinary *B. coli*, or haemolytic faecal strains, has been the formation of *local abscesses*.

(10) Feeding experiments with live cultures of haemolytic colon bacilli failed to produce a morbid process.

(11) The "sugar" reactions have proved of no value in the grouping of colon bacilli.

(12) Saturation of anti-coli sera with specific *B. coli* emulsions and other colon bacillus emulsions has assisted in the grouping of these organisms.

(13) We failed to observe any advantage in "massive doses" of *B. coli* in the immunisation of animals.

(14) In vaccine treatment in the human subject, it is necessary to investigate the organism isolated from the infected urine, and the blood serum of the patient, along the lines indicated by us. The methods for the administration of the vaccine must also be considered.

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THE STERILIZATION OF EMPTY MILK CHURNS BY STEAM UNDER PRESSURE¹.

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IN a previous paper² it was shown that of the empty milk churns which were examined, 56 per cent. were totally unfit for use, as they contained very large numbers of bacteria and lactose fermenting organisms in high proportion. Table I (1) is taken from this paper and gives the number of bacteria per 1 c.c. of washings with sterile saline solution. These churns were taken at random on a station platform, their origin being unknown.

Table I.

Bacteriological examination of empty milk churns.

(1) On a station platform.

Condition of churn	Bacteria per 1 c.c. washings	Lactose fermenting organisms
Unwashed	Uncountable in	
	1/100,000 c.c.	+ 1/100,000 c.c.
"	4,000,000	+ 1/100,000 "
Badly washed (milk and water present)	18,400,000	+ 1/1000 "
" " "	5,300,000	+ 1/1000 "
Apparently clean but wet	4,100,000	+ 1/100 "
" "	780,000	+ 1/10,000 "
Apparently clean and dry	6,200,000	+ 1/1000 "
" "	1,720,000	+ 1/10,000 "

(2) On a farm attempting to produce clean milk.

Apparently clean and dry	1,500,000	+ 1/10 "
" "	1,000,000	+ 1/100 "
Wet.—Foul smell	38,000	+ 1/10 "
" "	1,200,000	+ 1/10 "

The unsatisfactory condition of these particular churns may have been due to the lack of care on the part of the dealers from whom they happened to have come.

¹ The expenses of this investigation were defrayed by a grant from the Ministry of Health.

² Cumming and Mattick, 28. vii. 1920. An Enquiry concerning the State of Cleanliness of Empty Milk Churns. *Journ. of Hygiene*, xix.

That this fault is however general is well shown by a consideration of the lower set of figures in Table I (2), which gives the results of the bacteriological examination of churns used by a wholesaler who was undoubtedly endeavouring to assist the farmer in the production of clean milk.

These figures demonstrate that although the dealer was aware of the necessity for care and further that steam was available and had been used, it had not been employed to the best advantage.

It would seem, therefore, that there is at present no real conception of the best methods of cleansing milk churns in the industry.

CONDITIONS OF EXPERIMENT.

The following experiments have been carried out in order to discover the minimum time of steaming which is necessary to ensure as complete a sterilization as is possible under commercial conditions.

The steam used in the experiments was obtained from a boiler of a type which is in common use in the industry. The boiler worked at a pressure of from 40–80 lbs. and yielded steam at temperatures which varied, in the course of the experiments, from 89° C.–105° C. at the jet.

It should be noted that these variations of temperature and pressure and, what is probably most important, of the volume of steam, were accepted without question as they are conditions common to the open jets in general use.

It may be that a further study of the conditions governing these variations and their significance in sterilization may result in a considerable saving of time and expense involved in getting steam at an unnecessarily high pressure.

The experiments about to be described are divided into three sections in which the times of steaming are varied.

SERIES I.

Seventeen-gallon churns artificially contaminated with dirty milk.

Two 17-gallon churns in good condition, but not new, were contaminated by pouring into each two quarts of dirty milk.

Milk such as this was used so that the time of steaming finally arrived at should apply to any conditions likely to be found in practice.

The condition of this milk is shown by the results of the bacteriological examinations which were done at intervals and are tabulated in Table II.

The churns, with their lids on, were left with the milk in them, in an exposed place for 24 hours. At the end of this time the milk was poured out and the churns were washed.

Method of washing and steaming.

The bulk of the contaminating material, remaining in the churns, was removed by washing with a jet of cold water from a hose. Two gallons of cold water were then put in and the internal surface of each churn vigorously

scrubbed with a long handled brush resembling a deck swab ("Turk's Head"). This water was poured out and replaced by a similar quantity of hot water and the brush again used. The churns were then inverted to allow of drainage and finally steamed. One was steamed for thirty seconds and the other for three minutes. After steaming each churn was laid on its side to allow of the escape of steam.

It was found that the churns in this series and throughout the experiments which were steamed for three minutes were almost invariably quite dry after all the steam had escaped, there being sufficient residual heat in the metal to evaporate any water which might have condensed. This is an important point as it is sometimes considered necessary to employ hot air blasts etc. in order to dry churns after steaming.

After steaming, the lids, having received treatment similar to the churns, were replaced and the churns were left with their lids on in an exposed place for 24 hours as before.

Methods of making the bacteriological examinations.

At the end of this time, the internal surfaces of both churns were thoroughly washed by pouring in one litre of sterile saline solution and by rubbing vigorously with a sterile swab to detach bacteria from the sides. A part of the saline was first poured on to the inside of the lid and then allowed to run into the churn.

Samples of the washings from each churn were then taken into sterile bottles and at once examined bacteriologically by plating dilutions from 1/10–1/10,000 c.c. on neutral whey agar plates. These were incubated for five days at 22° C. and then counted.

Litmus lactose peptone water tubes were also inoculated with quantities of the washings varying from 1 c.c.–1/10,000 c.c. and incubated for five days at 37° C. The tubes were then examined for the presence or absence of lactose fermenting organisms as indicated by the production or non-production of acid and gas in the Durham's tubes.

Results of experiments in Series I.

The results, which are shown in Table II, demonstrate the fact that 30 seconds' steaming does not suffice to sterilize a churn which has been contaminated with such milk as was used. The counts obtained were irregular and on six occasions out of fifteen, lactose fermenting organisms were found to be present. When the churns had been steamed for three minutes the counts were usually very small and in some cases no organisms grew. This fact should not be taken to mean that no organisms were present, but simply that they were not found in the quantities examined and that in any case the numbers must have been very small.

The presence of lactose fermenting organisms was not demonstrated in those churns which had been steamed for three minutes.

Table II.

Seventeen-gallon churns artificially contaminated with sour milk.

Date	30 seconds' steaming		3 minutes' steaming	
	Agar count	Lactose fermenting organisms	Agar count	Lactose fermenting organisms
24. vi. 20	100	—	200	—
28. vi. 20	200	—	0	—
30. vi. 20	500	—	330	—
3. vii. 20	180	—	70	—
5. viii. 20	30	—	20	—
18. viii. 20	250	+	20	—
21. viii. 20	240	+	10	—
24. viii. 20	1520	+	0	—
27. viii. 20	0	—	10	—
4. ix. 20	400	+	10	—
8. ix. 20	620	+	370	—
18. ix. 20	30	—	20	—
23. ix. 20	30	—	20	—
25. ix. 20	142	—	12	—
1. x. 20	14700	+	7900	—

Examination of contaminating material.

23. vi. 20	Uncountable in 1/1,000,000 c.c.	+ 1/1,000,000 c.c.	.	.
2. vii. 20	Uncountable in 1/1,000,000 c.c.	+ 1/1,000,000 c.c.	.	.
26. viii. 20	Uncountable in 1/10,000 c.c.	+ 1 c.c.	.	.

SERIES II.

Seventeen-gallon churns artificially contaminated with sour whey.

Since 30 seconds' steaming had been proved to be inadequate to sterilize a heavily contaminated churn and three minutes' steaming appeared to give very satisfactory results, it was thought wise to try and discover the efficiency of a steaming time which was more than thirty seconds but less than three minutes.

A series of experiments was therefore set up in which the churns were steamed for periods of thirty seconds and two minutes respectively, after contamination with whey.

A period of thirty seconds' steaming was again used in this series so as to confirm the results of the former series.

The methods of washing the churns and carrying out the bacteriological examinations were exactly as before.

Results of experiments in Series II.

Forty-six experiments were made in this series, twenty-three after 30 seconds' steaming and twenty-three after two minutes' steaming.

Table III shows the results of the bacteriological examinations.

Table III.

Seventeen-gallon churns artificially contaminated with sour whey.

Date	30 seconds' steaming		2 minutes' steaming	
	Agar count	Lactose fermenting organisms	Agar count	Lactose fermenting organisms
3. xi. 20	8	—	4	—
9. xi. 20	0	—	1	+
11. xi. 20	160	+	40	—
13. xi. 20	30	—	100	+
16. xi. 20	1600	+	560	+
18. xi. 20	29	—	1	—
20. xi. 20	17	—	10	—
23. xi. 20	84	+	11	—
25. xi. 20	134	+	97	—
27. xi. 20	530	—	1580	+
30. xi. 20	41	—	2	—
2. xii. 20	188	+	10	+
4. xii. 20	138	—	6	—
7. xii. 20	740	+	540	—
9. xii. 20	720	—	9	—
11. xii. 20	12	—	2	—
27. i. 21	22200	—	2640	—
3. ii. 21	1430	—	190	—
5. ii. 21	121	—	14	—
8. ii. 21	760	—	57	—
10. ii. 21	Uncountable	+	140	—
12. ii. 21	101	—	22	—
17. ii. 21	2920	—	370	—

Examination of contaminating material.

8. xi. 20	Uncountable	+ 1/1000 c.c.	.	.
7. xii. 20	Uncountable	+ 1/1,000,000 c.c.	.	.

The bacterial counts obtained in both cases were found to be low but occasionally large numbers of bacteria were present.

The counts from the churns steamed for two minutes show, however, considerably fewer fluctuations than those from the one steamed for 30 seconds, and the numbers of occasions when lactose fermenting organisms were present, are less in the case of the former than of the latter.

It is seen that although two minutes' steaming gave better results than 30 seconds' steaming, there was still room for improvement.

These two series of experiments demonstrated that a steaming period of 30 seconds was insufficient but left the adequacy of two minutes' steaming still in doubt. A further series of experiments was therefore set up to establish the relative efficiencies of steaming for periods of two and three minutes.

SERIES III.

Seventeen-gallon churns contaminated with sour whey.

In this series a much greater quantity of whey was used for contaminating the churns, from four to five gallons being used for each.

Four 17-gallon churns were contaminated in this way and left for 24 hours in an exposed position with the lids on.

The methods of washing, steaming and making the bacteriological examinations remained unchanged, except that 1 c.c. of the washings was inoculated into neutral whey agar plates in addition to dilutions of 1/10 c.c. and upwards. Two churns were steamed for two minutes and two for three minutes at each examination.

Results of examinations in Series III.

Forty-four examinations were made under the above conditions, twenty-two after two minutes' steaming and twenty-two after three minutes' steaming. The results are tabulated in Table IV.

Table IV.

Seventeen-gallon churns heavily contaminated with sour whey.

Date	2 minutes' steaming		3 minutes' steaming	
	Agar count	Lactose fermenting organisms	Agar count	Lactose fermenting organisms
26. iv. 21	9	—	96	—
	11	—	50	—
28. iv. 21	350	—	27	—
	Uncountable	+	Uncountable	—
30. iv. 21	2150	—	38	—
	480	—	74	—
3. v. 21	115	—	8	—
	15	—	16	—
5. v. 21	27	—	12	—
	20	—	29	—
7. v. 21	46	—	6	—
	110	—	138	—
10. v. 21	10	—	1	—
	2	—	7	—
12. v. 21	12	—	15	—
	4	—	175	—
19. v. 21	20	—	7	—
	30	—	5	—
21. v. 21	2	—	50	—
	30	—	27	—
24. v. 21	5	—	6	—
	26	—	30	—

Except on one occasion (28. iv. 21) the actual counts were extremely low, there being very little to choose between the two sets of results.

It is, however, notable that on no occasion were lactose fermenting organisms found after three minutes' steaming, but that they were present on one occasion after two minutes' steaming.

SUMMARY OF RESULTS.

The results of all the experiments are summarized in Table V.

Table V.

Time of steaming	No. of experiments	500 bacteria or less per 1 c.c. of washings	Percentage of counts under 500 per 1 c.c. of washings
30 seconds	38	26	68 %
2 minutes	45	39	86 %
3 minutes	37	35	94.6 %

		Number of occasions when lactose fermenting organisms were present	Percentage of occasions when lactose fermenting organisms were present
30 seconds	38	13	34 %
2 minutes	45	6	13.3 %
3 minutes	37	0	0 %

In constructing Table V all those results giving 500 or less bacteria per 1 c.c. of washings are shown as percentages of the total number of experiments in the series. This figure (500 per 1 c.c.) was adopted for comparison with the figures shown in Table I. .

The figures in Table V clearly demonstrate the fact that as the time of steaming increases, the number of bacteria finally found in the churn decreases. When the time of steaming was 30 seconds only 68 per cent. of the churns examined showed counts as low as 500 or less as compared with 86 per cent. after two minutes' and 94.6 per cent. after three minutes' steaming.

The superiority of a period of three minutes' over two minutes' steaming is further clearly shown in Table V by a consideration of the percentages of occasions when lactose fermenting organisms occurred. After 30 seconds' steaming lactose fermenting organisms were found on 34 per cent. of occasions as compared with 13 per cent. after two minutes' steaming and 0 per cent. after three minutes' steaming.

It is of interest to compare the initial contamination of milk put into a properly cleansed churn with that of milk put into an inadequately cleansed churn.

One of the churns in Table I contained 18,400,000 bacteria per 1 c.c. of washings. This figure represents an initial contamination of any milk put into such a churn of 240,000 per 1 c.c. if 17 gallons of milk be introduced.

If, as in the churns of Series III, there are 500 bacteria per 1 c.c. of washings, then 17 gallons of milk would be contaminated to the extent of about 7 bacteria per 1 c.c.

The difference in these figures probably represents many hours difference in the duration of sweetness of the milk.

CONCLUSIONS.

Churns and other milk utensils, if they be dirty, are responsible for a great deal of unnecessary contamination in market milk. Any extra care taken in adequately cleansing churns would be more than repaid by the improvement in the quality of the milk.

The experiments show that if dirty churns be properly washed and then steamed for three minutes they can be used with safety after 24 hours, if the lids are not removed.

This is a point of great importance since the practice of many farmers, of swilling out churns immediately before use, with water which is often open to suspicion, becomes unnecessary.

In depots, where large numbers of churns have to be dealt with, it should be feasible to arrange for the installation of a greater number of steam jets. Since it takes three minutes to steam a churn adequately, each jet would be capable of dealing with 20 churns per hour.

Serious consideration should be given to the desirability of the provision of a duplicate set of churns, since under the present conditions, where lorries wait to return churns to the station, it is impossible to sterilize them properly.

It is undoubtedly the business of the persons receiving the milk to see that the churns are properly sterilized, as the difficulty of cleansing them after milk has been allowed to sour in them is very greatly increased.

Thanks are due to Miss Erskine for permission to use the figures in Table I (2).

AIR CARRIAGE OF PATHOGENIC AND OTHER ORGANISMS.

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IN all laboratories engaged in the preparation of serum the latter occasionally becomes contaminated by micro-organisms. It is often a matter of considerable difficulty to determine the origin of such organisms, and investigations upon the source of these contaminations not infrequently proves of considerable interest. This paper records some preliminary experiments which were designed to throw light on the subject.

The first experiments were made at a stable containing 20 horses, arranged in two equal rows separated by a wide central passage running from north to south and opening at each end of the building.

Agar plates were exposed for a period of 30 minutes as follows: six inside the stable, six, arranged in pairs, 30 feet from the open stable doors, the centre pair being opposite to the entrance with a space of 20 feet on either side between this pair and the lateral pairs.

Similar sets of six were placed at 90 feet and at 150 feet from the stable doors and control plates were placed in an open space 400 feet from any building.

The exposures were made on three dry summer days under the following meteorological conditions:

Day	Velocity of wind	Wet bulb	Dry bulb
1	normal	58° F.	60° F.
2	moderate	56° F.	58° F.
3	strong breeze	58° F.	60° F.

The table on p. 174 gives the results of the bacteriological examination of the plates after 18 hours' incubation at 37° C. The number of colonies represents the average of the six plates in each position.

The average number of colonies on the agar plates exposed at a distance of 400 feet from any building on various days was found to be six, most of them being staphylococci. Staphylococci, various species of mould and *B. subtilis* are commonly found in the fodder and bedding of the large herbivora and these organisms were presumably air borne directly from the stables.

In estimating the importance of any suspected source of bacterial contamination two methods are available, namely the identification of the bacterial population on the plate with that present at the source and the consideration of curves based upon the bacterial count obtained from plates

Calm day.

Situation	No. of colonies	Organisms found
Inside stable	300	Staphylococci, moulds, gram-negative bacillus, <i>B. subtilis</i>
30 ft. from stable	30	Staphylococci, moulds, gram-negative bacillus
90 " "	12	Staphylococci, moulds
150 " "	3	Staphylococci

Moderately windy.

Inside stable	800	—	—
30 ft. from stable	55	—	—
90 " "	23	Not ascertained	
150 " "	16	"	

Windy day.

Inside stable	730	Staphylococci, moulds, gram-negative bacillus, <i>B. subtilis</i>	
30 ft. from stable	200	" "	
90 " "	90	Staphylococci, moulds, <i>B. subtilis</i>	
150 " "	40	Staphylococci, moulds	

exposed at various points in relation to the source. The point at which the curve begins to "flatten out" may be regarded as the limit of contamination from the particular point, under the conditions of the experiment.

PATHOGENIC ANAEROBES IN THE AIR OF STABLES, ETC.

Petri dishes containing nutrient broth were exposed in a stable for a period of six hours, on each of six successive days. The six samples after being mixed were heated at 70° C. for 30 minutes. The heated broth was then inoculated into meat tubes and incubated at 37° C. for 24 hours. Microscopical examination of the resulting cultures revealed an apparently pure growth of a gram-positive, non-motile bacillus of from 5–8 microns in length and about 1–5 microns in breadth. Subsequent cultivation in milk produced the stormy fermentation characteristic of *B. welchii*. A small quantity of the culture was inoculated into three groups of guinea-pigs which had previously been injected with antisera to *B. welchii*, *Vibrio Septique* and *B. oedematiens* respectively, as well as into normal guinea-pigs. All the inoculated animals died of typical gas gangrene with the exception of those which had previously received an injection of *B. welchii* antiserum. From these experiments it is clear that *B. welchii* was commonly present in the air of the stable.

Six samples of dust from the interior of the roof of a second stable and six samples of material from crevices of the floor were collected in separate sterile vessels, and portions of each were inoculated into meat tubes which were incubated under anaerobic conditions, at 37° C. from 18 to 24 hours. The presence of *B. welchii* in every tube was established by the method already described.

Similarly this organism was proved to be present in the dust obtained in seven different buildings in which small laboratory animals (guinea-pigs and rabbits) were housed.

Since *B. welchii* apparently was constantly present in animal habitations it was decided to ascertain the distance that this organism might be carried, in the infected dust.

The experiment was performed as follows:

The direction of the prevailing wind was ascertained by means of thistle-down liberated from the top of a pair of steps about 7 feet from the ground.

Petri dishes containing nutrient broth were then placed at six points at distances of 30 feet and 90 feet radiating from the steps and in the direction of the wind current.

Some stable dust was then blown from the top of the steps by means of ordinary hand bellows.

The presence of *B. welchii* was established in each of the plates, except that on the extreme right at 90 feet, by cultural methods and animal experiments. Owing to lack of space the limits of dissemination of contaminated dust could not be ascertained.

SUMMARY.

From the foregoing experiments, it may be assumed:

(1) That given favourable conditions, such as a direct current of air through a building, organisms derived from animals, their fodder or environment, may be disseminated over distances up to 150 feet from the building.

(2) That *B. welchii* is frequently present in the dust and dirt of buildings in which horses and small laboratory animals (guinea-pigs and rabbits) are housed, and

(3) That under suitable conditions, spore-bearing anaerobes such as *B. welchii*, may be carried with infected dust from such buildings, for a distance of at least 90 feet, and probably considerably further.

NOTES ON THE PRODUCTION OF IMMUNITY TO DIPHTHERIA TOXIN.

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(With 15 Charts.)

CONTENTS.

	PAGE
INTRODUCTION	178
PART I. INJECTION OF TOXIN INTO ANIMALS WITH	
NO NORMAL ANTITOXIN	182
A. INJECTION OF TOXIN ALONE	182
<i>Guinea-pigs</i>	182
(1) Sublethal doses of toxin increase the susceptibility of guinea-pigs to subsequent intoxication.	
(2) Guinea-pigs may be killed by less than 1 m.l.d. if small doses are given at frequent intervals. The effect produced by dividing the injection depends upon the size and the time interval of the dose.	
(3) Toxin rich in "toxoid" may produce high immunity.	
(4) Guinea-pigs have been immunised successfully on rare occasions by a series of injections of toxin alone.	
<i>Horses</i>	186
(5) Horses possessing no normal antitoxin resemble guinea-pigs in their reaction to toxin and in the difficulty of immunisation.	
B. INJECTION OF TOXIN UNDER COVER OF ANTITOXIN	186
(a) Antitoxin injected at the same time as toxin.	
<i>Guinea-pigs</i>	186
(6) A single injection of a toxin-antitoxin mixture may confer a high degree of immunity.	
(7) Comparatively little immunity is produced for at least four weeks after the injection of a toxin-antitoxin mixture and the maximum is reached at eight weeks or later.	
(8) Individual guinea-pigs vary considerably in their immunity response to an injection of a toxin-antitoxin mixture.	
(9) It would appear that a moderate excess of antitoxin in the immunising mixture does not affect materially the degree of immunity produced. A considerable excess of antitoxin is required to render a mixture incapable of producing immunity.	
(10) The immunity conferred by a single injection of a toxin-antitoxin mixture is of long duration.	
<i>Rabbits</i>	192
(11) Rabbits injected with a toxin-antitoxin mixture give results similar to those produced in guinea-pigs.	
(12) More toxic mixtures produce higher immunity.	
(13) Splenectomised rabbits respond to injections of toxin-antitoxin mixtures.	

- Other animals*
- (14) Goats injected with toxin-antitoxin mixtures give results similar to those produced in guinea-pigs.
- (15) Horses, possessing no normal antitoxin injected with toxin-antitoxin mixtures give results similar to those produced in guinea-pigs.
- (16) The amount of toxin injected, in relation to the size of the animal, is of importance in the production of immunity.

(b) Antitoxin injected before toxin.

- Guinea-pigs* 196
- (17) Immunity may be produced by an injection of toxin into guinea-pigs that have previously received heterologous antitoxin; the results are not uniform.

(c) Antitoxin present in the form of passive immunity transmitted from mother.

- Guinea-pigs* 199
- (18) A high degree of immunity sometimes results from a single injection of toxin in a guinea-pig passively immune by maternal transmission.
- (19) The amount of toxin injected must be large in proportion to the antitoxin present in order that immunity may result.
- (20) Comparatively little immunity is produced during the first four weeks after the injection.

(21) *Summary of Part I.*

The primary stimulus in guinea-pigs, rabbits, goats and horses is followed by a latent period of about three weeks, and the maximum immunity is reached in about eight weeks.

PART II. INJECTION OF TOXIN INTO ACTIVELY IMMUNE ANIMALS. . . 201

Guinea-pigs 201

- (22) An injection of toxin into an actively immune guinea-pig produces far greater immunity than an injection into a passively immune guinea-pig.
- (23) A marked increase of antitoxin occurs within a few days of an injection of toxin into an actively immune guinea-pig and the maximum is reached in about 12 days.
- (24) The degree of immunity produced depends partly upon the constitution of the original immunising mixture.
- (25) Relatively high immunity similarly results from the injection of toxin-antitoxin mixtures into actively immune guinea-pigs.
- (26) Two injections of a neutral mixture into normal guinea-pigs do not produce any higher immunity than a single injection unless sufficient time elapses between the two injections.
- (27) If a long interval of time (a year or more) elapse before the second injection, high immunity is likewise produced.

Rabbits 207

- (28) The injection of toxin into actively immune rabbits gives results similar to those obtained with guinea-pigs.

Sheep and Goats 210

- (29) A rapid formation of antitoxin occurs after the injection of toxin into actively immune sheep and goats.

Horses 211

- (30) The second injection of a toxin-antitoxin mixture causes a rapid production of antitoxin in horses possessing no normal antitoxin.
- (31) The response to a second injection rapidly following the primary stimulus in a horse possessing no normal antitoxin is of an intermediate character between the usual responses following the primary and the secondary stimulus.

(32) *Summary of Part II.*

The secondary stimulus in guinea-pigs, rabbits, goats, sheep and horses is followed by a latent period of about four days and the maximum immunity is reached in about ten days.

PART III. INJECTION OF TOXIN INTO ANIMALS

POSSESSING NORMAL ANTITOXIN 215

Horses 215

- (33) The injection of toxin into horses possessing normal antitoxin produces a rapid increase in antitoxin.
- (34) The injection of toxin into horses possessing very little immunity acts as an intermediate stimulus.
- (35) A toxin-antitoxin mixture may produce considerable immunity in a naturally immune horse.

Humans 219

- (36) The injection of a toxin-antitoxin mixture into a naturally immune human causes a rapid production of antitoxin.

(37) *Summary of Part III.*

The injection of toxin into a naturally immune horse and human acts as a secondary stimulus and is followed by a latent period of about four days, and the maximum height of immunity is reached in about ten days.

- (38) The injection of toxin into a "partially" immune animal acts as an intermediate stimulus.

INTRODUCTION.

THE present paper deals with some of the results of our work on immunity, extending over a period of many years.

During the period occupied by this research, publications have appeared at various times dealing with similar lines of work or reaching similar conclusions. We, however, do not consider that any part in which conclusions agree with those already published should be omitted, since each section of the paper has a bearing on some other section.

Though we do not bring forward anything that is fundamentally new, we believe that, on many points and particularly in connection with what we have called the "Secondary Stimulus Phenomenon," our observations have attached a clear and quantitative value to an idea that is foreshadowed in several places in the literature of immunity, but of which we cannot find a clear description in any of the modern text-books.

A review of the field of work covered will be of more general interest, for a full knowledge of this field has a very definite practical bearing on such problems as those involved in the active immunisation by toxin-antitoxin mixtures of people susceptible to diphtheria, in the protection of horses exposed to the risk of tetanus by mixtures of tetanus toxin and antitoxin, the long protection afforded by vaccination with vaccine lymph and by typhoid vaccine, etc.

Review.

The chief point of interest in this paper is the marked contrast between the immunity response to the primary stimulus and the response to the secondary stimulus.

Primary Stimulus is the term that we apply to the initial injection of an antigen into a non-immune animal. The primary stimulus causes a very slow production of antibody; so delayed is the response that many weeks may elapse before the presence of antitoxin can be detected in the circulating blood.

In no instance have we detected an immunity response in less than two weeks after the primary stimulus nor has the highest immunity been attained in less than five weeks. The average length of latent period following a primary stimulus is three weeks and the maximum immunity is usually reached in about eight weeks (see statement 21 and Table XXIV).

Secondary Stimulus is the term that we apply to the injection of toxin into an actively immunised animal. In contrast to the primary stimulus this causes a rapid response, the latent period is about four days and the maximum immunity is reached in about ten days (see statement 32 and Table XXXV). The secondary stimulus phenomenon has been demonstrated in guinea-pigs, rabbits, sheep, goats and horses. The injection of toxin into naturally immune horses and into one naturally immune human was followed by a similar response to that caused by a secondary stimulus in an animal artificially immunised by a primary stimulus (see statement 37 and Table XLI).

That certain animals in domestication or in intimate association with man, such as the horse, frequently possess diphtheria antitoxin in their blood, while other animals, such as the guinea-pig and the rabbit, do not possess antitoxin, are now well-known facts.

It has also been established that a large proportion of human beings who have never had clinical diphtheria, possess normal antitoxin in their blood. The question of the origin of diphtheria antitoxin in the normal individual is obscure and therefore the subject of theoretical speculation. Some would hold that its possession is an innate quality and hereditarily transmitted, while others, basing their opinion on the finding of the diphtheria bacillus in healthy people, and at the seat of superficial ulceration in horses, believe that diphtheria antitoxin in the healthy normal individual has arisen in all cases in direct response to the presence of the diphtheria bacillus at some time or other during life, although its presence may have given rise to little or no appreciable alteration in the normal condition of health.

By comparison of the tables and curves, it will be seen that the behaviour of animals possessing normal antitoxin, to an injection is the same as that of animals previously immunised by the injection of the specific toxin. This gives strong support to the theory that the origin of normal antitoxin is by active response at some time or other to an infection by the diphtheria bacillus.

That natural immunity is an active immunity produced by a natural infection has long been accepted although it has been difficult to account for the natural immunity present in the majority of horses. The only recorded case of *B. diphtheriae* found in a horse was that by Cobbet (*Lancet*, 25 Aug.

1900) until very recently when Minett found the organism in limb lesions of a number of horses (*Journal of Comparative Pathology*, Dec. 1920).

Intermediate Stimulus is the term that we apply to the injection of toxin into an animal that is only partially immune. It is obvious that there cannot be a sharp division between normal animals capable only of a primary response and actively immune animals fully equipped for a secondary response. A second injection of toxin given when the primary response is commencing to develop is followed by an immunity response intermediate in character between primary and secondary responses (see statement 38 and Table XLII).

The quantity of toxin that can be injected without injury to a non-immune animal is very small; in order to increase the primary stimulus it is necessary therefore to use toxin the lethal power of which has been weakened by age, by chemical agents such as iodine or formalin, or almost completely neutralised by antitoxin. The effect of certain chemical agents is to destroy the lethal power without altering appreciably the antigenic value of a toxin. Under statement 3 in Part I A there are recorded the only instances in this paper in which such a toxin has been used. In the majority of cases the primary stimulus has consisted of a toxin-antitoxin mixture and the secondary stimulus of a greater amount of toxin without antitoxin. The difference in response to the two stimuli is not due to the difference in intensity of stimulus. Theobald Smith (*Journal of Experimental Medicine*, 1909) has shown that greater immunity is produced by a single injection of multiple doses of a toxin-antitoxin mixture than by a single dose; that this does not account for the difference between the primary and secondary phenomena is shown in Table XXXIII where both primary and secondary stimuli consisted of toxin-antitoxin mixtures of identical composition, and in Table XXVII where the secondary stimulus consisted of a less toxic mixture than the primary stimulus. It is at present a matter of theoretical speculation as to the amount of toxin in a toxin-antitoxin mixture that is available as an antigen. Mixtures lying within the "Differential Region" (Ehrlich) or within the "visible spectrum of the toxin-antitoxin effects" (Theobald Smith) contain uncombined toxoid and some antigenic power is thus explicable; why completely neutralised and over neutralised mixtures also act as antigens is less clear. There appear to be three possible explanations, that the combined toxin-antitoxin complex acts in itself as an antigen, that dissociation occurs between the toxin and antitoxin, or that there is present a variety of toxoid uncombined that lies outside the visible spectrum.

Instances of the negative phase are seen in Tables XXII, XXXI, XXXVI, XXXVIII. The negative phase is distinct from the latent period and consists of a fall in antitoxic value after a secondary stimulus greater than can be accounted for by combination between the circulating antitoxin and the injected toxin.

The primary and secondary stimulus phenomena have been demonstrated in this paper for guinea-pigs, rabbits, goats, horses and humans injected with

diphtheria toxin. Similar phenomena occur in animals injected with the toxins of the pathogenic anaerobes (work yet to be published by one of us). The response of many different animals to five different toxins follows the same definite course; the primary and secondary stimulus phenomena may yet be found of universal application to immunity and not limited only to antitoxin production.

Methods.

Except where otherwise stated, Toxin 1915 was used throughout. The m.l.d. of this toxin was 0.005 c.c. and its L0 and L + doses were 0.32 c.c. and 0.38 c.c. respectively.

Toxin J 176 is occasionally referred to; this toxin is of considerable interest as the L + dose has remained unchanged for 13 years. The toxin has been kept in the liquid form in an ice chest and has been in constant use. Under certain conditions, therefore, diphtheria toxin may remain stable.

The results of any injections into an animal are recorded by noting the day of death, or, if the animal survives for five days, by noting the size of the local reaction and the loss or gain in weight during the five days following the injection. The size of the local reaction is classified as follows:

No reaction	nil	Medium swelling	MS
Very small swelling	trace	Large swelling	LS
Small swelling	SS	Very large swelling	VLS

The numbers following the size of the swelling indicate in grammes the rise or fall in weight during the five days following the injection.

When only small quantities of blood were available for examination as from the ear of a guinea-pig, we invariably collected the blood in a Thoma Zeiss red corpuscle counting pipette which afforded a convenient apparatus for the accurate measurement of small volumes. A 4 per cent. solution of sodium citrate was drawn up into the pipette directly after the required volume of blood. The contents were then blown into a small glass containing the dose of toxin previously made up to 4 c.c. by the addition of normal saline and about 0.15 c.c. of sodium citrate solution. The pipette was washed out by drawing the fluid up and blowing out a few times. The blood and toxin were mixed by gentle shaking. We found that a much larger quantity of sodium citrate than that used in testing by this method had no influence on either toxin or antitoxin. A series of control experiments was made by injecting a number of guinea-pigs with varying amounts of antitoxin mixed with different fractions of a test dose of toxin. From the table of results obtained it was possible to interpolate intermediate results and to interpret the results obtained with test animals in terms of fractions of a unit of antitoxin in the blood tested. When only small quantities of blood were available the smallest amount of antitoxin that could be detected was 0.04 unit per c.c., only values greater than this can be accepted with any confidence. When testing larger animals such as the horse not only was more serum available

for each test but a larger number of tests could be made upon the serum obtained from each test bleeding; it follows that such results are more accurate and a smaller amount of antitoxin could be detected. Values of 0.01 unit per c.c. are however of doubtful significance. It might be pointed out here that since this work was done it has been found possible to test for as little as 0.0005 unit per c.c. by means of the intracutaneous method.

Arrangement of results.

The results are divided into three parts:

Part I. Injection of toxin into animals with no normal antitoxin.

A. Injection of toxin alone.

B. Injection of toxin under cover of antitoxin.

Part II. Injection of toxin into actively immune animals.

Part III. Injection of toxin into animals possessing normal antitoxin.

In each part the results with different species of animal are given separately. For the sake of ease of reference each result is prefaced by a statement of the fact subsequently demonstrated by the results recorded. An index of parts, subdivisions and statements is given at the beginning of the paper.

PART I.

INJECTION OF TOXIN INTO ANIMALS WITH NO NORMAL ANTITOXIN.

A. INJECTION OF TOXIN ALONE.

Guinea-pigs.

(1) *Sublethal doses of toxin increase the susceptibility of guinea-pigs
to subsequent intoxication.*

A number of guinea-pigs that had survived sublethal doses of toxin were injected with 1 m.l.d. of toxin from 3-8 weeks later when they had regained approximately their original weight. It was found that the majority of those that had been seriously affected by the original injection died within three days. Normal guinea-pigs injected with the same dose of toxin usually died upon the fifth day; as will be seen from Table I, only 16 per cent. died on the

Table I.

The effect of a single fatal dose of toxin upon guinea-pigs previously
injected with a sublethal dose of toxin.

	Total tested	Number dying within three days	Percentage number of early deaths
Normal guinea-pigs during corresponding period ...	19	3	16
Guinea-pigs previously injected with toxin causing loss in weight during the first five days of less than 30 grms.	10	2	20
30-40 grms.	16	4	25
40-50 grms.	7	2	28
Over 50 grms.	9	8	89

third day or earlier, as compared with 89 per cent. among those that had been severely affected by the first injection.

Among other guinea-pigs which had increased in weight during the first five days after the first injection, a subsequent injection of 1 m.l.d. of toxin, 3-8 weeks later, gave the same results as in normal animals.

An increase in susceptibility after an injection of toxin altered to toxoid or in combination with antitoxin is also seen in Tables III and VI.

It is open to question whether this increased susceptibility is really specific. It is possible that, had the first injection been any other poison, *e.g.* tetanus toxin or a poisonous drug producing the same general lowering of vitality, similar increased susceptibility to a subsequent injection of diphtheria toxin would have been met with.

- (2) *Guinea-pigs may be killed by less than 1 m.l.d. if small doses are given at frequent intervals. The effect produced by dividing the injection depends upon the size and the time interval of the dose.*

The results of a number of experiments are given in Table II.

Table II.

The effect of injecting fractions of a lethal dose of toxin
at different intervals.

Dose given at each injection	Results obtained from		Fraction of normal m.l.d. required to kill the animal when injected	
	Daily injection	Injection every two days	Daily	Every two days
2/5 m.l.d.	—	Died after 2 inj.	—	4/5
1/5 „	Died after 4 inj.	1 died after 5 inj. 3 „ 7 „	4/5	7/5
1/10 „	4 died after 6 inj. 1 „ 7 „ 2 „ 10 „ 1 survived 10 „	1 „ 12 „ 3 survived 12 „	about 8/10	greater than 12/10
1/20 „	1 died after 16 inj. 2 „ 24 „ 1 survived 24 „		about 24/20	

The number of experiments is very small but there are indications to show that, in order to kill a guinea-pig with a total quantity of less than the minimal lethal dose, an interval of two days may elapse between the injections when 2/5 of an m.l.d. is given at each injection; when 1/5 or 1/10 m.l.d. is given, the result is only brought about at a shorter interval of time and one day may elapse between the injections; again, when 1/20 m.l.d. is given at daily intervals, three out of four animals survive a total above the m.l.d. It probably follows that if an injection of 1/20 m.l.d. or less were given more frequently than at daily intervals, death would follow before an amount equal to 1 m.l.d. had been given. The intervals of time between the injections must be spaced according to the dose; they must be of longer duration when the dose is large but must not exceed a limit which becomes smaller as the dose is lessened.

The fact that very few experiments were performed under this section must again be stressed, but the indications are of sufficient interest to justify their inclusion in the paper.

From results given later, under statement 4, of attempts to immunise guinea-pigs by a series of injections of toxin alone, it will be seen that sub-lethal doses at weekly intervals are frequently fatal.

(3) *Toxin rich in "toxoid" may produce high immunity.*

The toxin used in this experiment had originally an m.l.d. of 0.005 c.c.; the toxin was changed into "toxoid" by treatment with formalin rendering the product atoxic, so that 5 c.c. would not kill.

Three weeks after a single dose of from 0.5 c.c. to 2.0 c.c. of an old formalinised toxin (L0 dose about 1.0 c.c.—m.l.d. over 5.0 c.c.) a number of guinea-pigs survived 2 m.l.d. of toxin without any local reaction.

Table III gives the results obtained by injecting four other guinea-pigs with increasing doses of the same formalinised toxin.

Table III.

The course of injection of four guinea-pigs with formalinised toxin.

Interval	Dose	Guinea-pig V	Guinea-pig X	Dose	Guinea-pig W	Guinea-pig Z
—	0.2 c.c.	SS + 20	trace + 15	0.5 c.c.	LS + 15	LS + 10
5 weeks	0.5 c.c.	SS - 10	LS - 5	1.0 c.c.	VLS - 25	LS - 20
2 „	1.0 c.c.	nil - 45	trace + 25	2.0 c.c.	nil + 40	nil + 50
2 „	2.0 c.c.	nil + 50	nil + 40	5.0 c.c.	nil + 40	trace + 65

N.B. For interpretation of symbols in this and all succeeding tables see explanation given under heading "Methods."

In all cases, two weeks after the last injection, antitoxin was demonstrated in the blood. *W* was the only animal whose antitoxic value was worked out and that was found to be 8.5 units per c.c. It will be noticed that in all cases the second injection was not tolerated so well as the first or subsequent ones. (See statement 1.)

(4) *Guinea-pigs have been immunised successfully on rare occasions by a series of injections of toxin alone.*

Attempts to immunise guinea-pigs against unmodified toxin gave variable results. In the majority of cases the animals failed to survive a long series of injections. Death may have been due to the poor condition of the guinea-pigs, the injections having rendered them more susceptible to adverse conditions. In a few cases, post-mortem examination showed that death was due to other causes than diphtheria intoxication.

Table IV records the results obtained by giving a number of guinea-pigs weekly injections of toxin (m.l.d. 0.005 c.c.).

In the case of guinea-pig *R* in which antitoxin was produced, the animal was immunised very slowly, no increase to 0.002 c.c. being made until after

ten injections at 0.001 c.c. (1/5 of m.l.d.). In another series, the animals were allowed to rest for nine weeks after the first injection and were then injected

Table IV.

The course of immunisation of six guinea-pigs receiving weekly injections of toxin, showing the number of weekly injections at each dose.

Dose	Guinea-pig A	Guinea-pig C	Guinea-pig F	Guinea-pig P	Guinea-pig R	Guinea-pig S
0.0005 c.c.	2	2	3	—	—	—
0.0006 c.c.	—	—	—	1	1	1
0.001 c.c.	8	4	1	9	10	9
0.002 c.c.	—	3	—	2	4	3
0.003 c.c.	—	—	—	—	2	—
0.004 c.c.	—	—	—	—	3	—
Result ...	Death	Death	Death	Death	Over 1 unit of anti-toxin produced	Death

weekly. A number of deaths occurred, but in two cases the immunisation was successful. These two cases are recorded in Table V.

Table V.

The course of immunisation of two guinea-pigs receiving weekly injections of toxin at a long interval after an initial injection.

S 2. iv.

0.0005 c.c. (1/10 m.l.d.). Reaction SS + 10 grms.

Interval nine weeks.

Three weekly injections of 0.001 c.c.

Nine weekly injections of 0.002 c.c.

Antitoxic value of blood tested nine days after the last injection = 1.4 units per c.c.

W 2. iv.

0.0005 c.c. (1/10 m.l.d.). Reaction—trace—no change in weight.

Interval nine weeks.

Three weekly injections of 0.001 c.c.

Nine weekly injections of 0.002 c.c.

Three weeks later 0.02 c.c. (4 m.l.d.).

Five days later 0.05 c.c. (10 m.l.d.). Reaction—nil + 50 grms.

Antitoxic value of blood not tested.

In a further series larger doses and longer intervals were employed. Two guinea-pigs out of a number survived the course of injections and showed marked immunity. From Table VI, where these cases are recorded, it will be seen that guinea-pig *P* 8. xii. produced 70 units of antitoxin per c.c. of blood and was able easily to tolerate 2000 times the m.l.d. of toxin.

The successful immunisation of the animals recorded in Tables V and VI was probably due to the fact that the main course of injection was started about two months after the first injection, at a time when the animals were actively immune (see statement 21).

Table VI.

The course of immunisation of two guinea-pigs receiving injections of toxin at long intervals.

P 8. xii.

Dose	Result	Interval	Result
0.005 (toxin J 2172)	MS + 25 grms.	7 weeks	—
0.003 (toxin J 1915)	LS - 30 „	3 „	No antitoxic value 10 days later
0.004 „	LS - 80 „	2 „	—
0.004 „	nil + 10 „	3 „	—
0.004 „	nil + 35 „	3 „	Antitoxic value detectable 4 days later
0.02 „	nil - 10 „	2 „	Over 8 units per c.c. 4 days later
0.1 „	nil - 15 „	2 „	—
10.0 (2000 m.l.d.)	trace - 20 „	—	About 70 units per c.c. 18 days later

S 15. iv.

0.001	trace + 30 grms.	9 weeks	—
0.002	VLS + 25 „	3 „	—
0.004	VLS + 55 „	5 „	—
0.004	- 65 „	2 „	—
0.004	- 5 „	5 „	—
0.02	+ 5 „	5 days	—
0.05 (10 m.l.d.)	nil	—	Antitoxic value not tested

Horses.

- (5) *Horses possessing no normal antitoxin resemble guinea-pigs in their reaction to toxin and in the difficulty of immunisation.*

In our experience in the routine immunisation of horses for the production of diphtheria antitoxic sera, horses (over seven years old) normally possessing no detectable antitoxin (less than 1/500 units per c.c.) in their serum are rare. Such horses are extremely difficult to immunise with toxin alone.

B. INJECTION OF TOXIN UNDER COVER OF ANTITOXIN.

- (a) **ANTITOXIN INJECTED AT THE SAME TIME AS TOXIN.**

Guinea-pigs.

- (6) *A single injection of a toxin-antitoxin mixture may confer a high degree of immunity.*

About 350 guinea-pigs that had been used for routine testing of antitoxin against toxin were subsequently tested for immunity by the injection of toxin. It was found that a number were able to tolerate as much as 400 times the m.l.d. for a normal guinea-pig. They had all received mixtures containing one test dose of toxin together with varying amounts over one unit of antitoxin. The animals were divided into two groups—those that had received mixtures causing local reactions, and those that had received mixtures in which the excess of antitoxin was sufficient to prevent local reaction. In the former group, the constitution of the test mixture only varied between the

narrow limits of the "differential region" L0 to L+; while in the latter group, there was an excess of antitoxin over that contained in the L0 mixture. The results of injecting toxin into guinea-pigs of the two groups are recorded in Tables VII and VIII. Owing to individual variability it is impossible to predetermine for any particular guinea-pig an exact dose at which a reaction will occur. Since only one test of tolerance can be made on one guinea-pig, it will be seen that the large number of guinea-pigs mentioned above was required in order to arrive at any conclusion by this method. The results are divided according to the number of weeks elapsing between the injection of the toxin-antitoxin mixture and the injection of toxin. The tables give the number of guinea-pigs surviving on the fifth day after the injection of toxin out of the total number injected with any particular dose. The figures printed in heavy type indicate the nearest dose, for the interval of time, at which approximately equal proportion of deaths and survivals occur and which can be taken as an index of tolerance.

Table VII represents the first group, *i.e.* those that had received mixtures causing local reaction.

Table VII.

Showing ratio of survivors to the total number of guinea-pigs injected with any particular dose of toxin, divided according to the interval of time elapsing after the injection of a toxin-antitoxin mixture causing local reaction.

Dose of toxin	Weeks elapsing between injection of toxin-antitoxin mixture and injection of toxin only														
c.c.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
5.0	0/5
2.0	7/14	.	.	.	7/14	.	.	0/2	1/1
1.0	1/6	.	5/5	.	.	.	4/5	.	.	1/4	.
0.5	.	.	0/6	.	5/10	.	6/8	.	.	.	4/5
0.2	.	.	1/3	.	5/8	.	2/2
0.1	.	.	2/14	.	.	.	2/2	.	.	.	2/2
0.064	.	.	11/18	.	.	.	4/4
0.032	0/1	.	7/11
0.016	.	6/16	10/13	.	1/1
0.008	.	9/13	4/5	.	4/4	.	2/2
0.005	15/18

Table VIII represents the second group, *i.e.* those that had received a mixture in which the excess of antitoxin was sufficient to prevent local reaction. The results of the injection of toxin into animals of this group are of less importance than those of the first group as in many instances a very large excess of antitoxin may have been present in the mixture, while in other cases an exact L0 mixture may have been injected. It is shown later (in statement 9) that a large excess of antitoxin in the mixture injected reduces the extent of immunity produced.

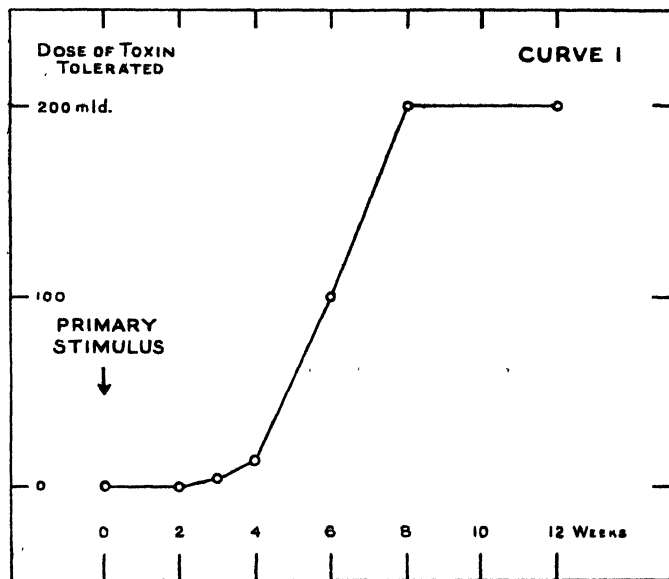
Table VIII.

Showing ratio of survivors to the total number of guinea-pigs injected with any particular dose of toxin, divided according to the interval of time elapsing after the injection of a toxin-antitoxin mixture causing no local reaction.

Dose of toxin	Weeks elapsing between injection of toxin-antitoxin mixture and injection of toxin only											
c.c.	2	3	4	6	8	12	16					
2.0	1/4	1/5	0/1
1.0	.	.	.	0/5	4/11	.	3/6
0.5	4/12	0/1
0.2	.	.	.	5/12
0.1	.	.	1/7
0.064	.	.	3/6	.	3/3
0.032	.	.	3/4
0.016	.	3/11	4/5
0.008	.	5/10	2/5	.	1/2
0.005	5/8

- (7) *Comparatively little immunity is produced for at least four weeks after the injection of a toxin-antitoxin mixture and the maximum is reached at eight weeks or later.*

The results in Tables VII and VIII show that a high degree of immunity, as shown by resistance to toxin, generally results from a single injection of a toxin-antitoxin mixture but the immunity is slow in making its appearance. It is of interest to note that two weeks after the injection of a mixture



CURVE I. From results given in Table VII showing average resistance of guinea-pigs to diphtheria toxin after an injection of a toxin-antitoxin mixture causing local reaction.

Latent period: 3 weeks. Maximum height: 8-12 weeks.

causing local reaction, some of the guinea-pigs injected will not tolerate 0.005 c.c. that is, the average m.l.d. for a normal 250 gm. guinea-pig, although these animals have increased in weight. The first two or three weeks may be considered as a latent period of no immunity. At the end of the third week, slight but definite immunity is shown, and after this it rises quickly to the sixth week, reaching its maximum at about the eighth week.

The average dose of toxin tolerated by guinea-pigs at different intervals of time after an injection of toxin-antitoxin mixture causing local reaction is shown in Curve 1.

A few guinea-pigs that had been used for routine testing of antitoxin and had shown local reaction as the result of the toxin-antitoxin mixture injected were tested for antitoxic value at various intervals of time after the injection. The results are recorded in Table IX.

Table IX.

The antitoxic value of the blood of guinea-pigs at different intervals after an injection of a toxin-antitoxin mixture.

Interval of time elapsing between toxin-antitoxin injection and testing of blood for antitoxin	Number of guinea-pigs tested	Antitoxic value of blood of individual guinea-pigs
4 weeks	2	0.0; 0.0 unit per c.c.
8 "	2	0.14; 0.14 unit per c.c.
12 "	5	0.07; 0.07; 0.14; over 0.15; 0.21 unit per c.c.

(8) *Individual guinea-pigs vary considerably in their immunity response to an injection of a toxin-antitoxin mixture.*

From Table VII, it will be noted that eight weeks after an injection of a toxin-antitoxin mixture, some animals (7 out of 14 tested) were found to tolerate 2.0 c.c. of toxin, while others (2 out of 8) were killed by one-quarter of the dose. Similar differences are shown at 12 weeks. At an earlier period, the differences are more marked, *e.g.* four weeks after the original injection, one guinea-pig out of three tested survived an injection of 2.0 c.c., while another guinea-pig (1 out of 5 tested) was killed with as small a dose as 0.008 c.c. (probably about the m.l.d. for a normal guinea-pig of the same weight). We are inclined to doubt whether such differences are to be ascribed to differences in the constitution of the toxin-antitoxin mixture which lay within the narrow range covered by the "differential region." Other individual differences are shown later in Table XI.

(9) *It would appear that a moderate excess of antitoxin in the immunising mixture does not affect materially the degree of immunity produced. A considerable excess of antitoxin is required to render a mixture incapable of producing immunity.*

A number of animals were injected with different mixtures containing one test dose of toxin and varying amounts of antitoxin from 1.2 units to

5.0 units. The mixture containing 1.2 units antitoxin and one test dose of toxin occasionally produced slight local reaction. This can be taken as being very close to the border of the L0 dose. At an interval of from 13 to 15 weeks, some of the guinea-pigs were injected with 2.0 c.c. (400 m.l.d.) of toxin. The results are given in Table X.

Table X.

Recording the number of guinea-pigs surviving 400 m.l.d. of toxin three months after the injection of a toxin-antitoxin mixture containing varying amounts of antitoxin.

Constitution of mixture		Number of guinea-pigs surviving 2.0 c.c. toxin	Number of guinea-pigs killed by 2.0 c.c. toxin
Toxin	Antitoxin		
1 test dose	1.2 units	3	1
1 "	1.3 "	1	2
1 "	1.5 "	4	1
1 "	2.0 "	0	4

From the small number of animals, it appears therefore that within the limits represented by 1.2 to 1.5 units of antitoxin in the mixture no marked differences are shown. A noticeable falling off is in evidence when the mixture contains 2.0 units of antitoxin, since all four guinea-pigs tested were killed by 2.0 c.c. of toxin. Such a mixture, however, produced quite definite immunity, for another guinea-pig tested survived an injection of 1.0 c.c. (100 m.l.d.) of toxin. A few guinea-pigs that had received mixtures over-neutralised to the extent of five units of antitoxin to one test dose of toxin, were injected with a single fatal dose of toxin and showed very little, if any, increased resistance.

The immunity conferred by toxin-antitoxin mixtures in guinea-pigs was also tested by ascertaining the antitoxic value of the blood of guinea-pigs after such an injection. The amount of antitoxin present was found to be extremely small and the results are somewhat inconsistent owing to the difficulty of testing small amounts of blood for traces of antitoxin.

The groups of guinea-pigs recorded above in Table X were tested for antitoxin at various times before the injection of toxin; it was found that little or no antitoxin was detectable four weeks after injection, and that for 2-3 months the highest values were obtained by the group injected with the mixture containing 1.2 units of antitoxin. The results are recorded in Table XI.

Table XI.

The antitoxic value of the blood of guinea-pigs 2-3 months after the injection of a toxin-antitoxin mixture containing varying amounts of antitoxin.

Constitution of mixture		Number of guinea-pigs showing			
Toxin	Antitoxin	0.00 unit of antitoxin	0.05	0.10	0.20
1 test dose	1.2 units	0	2	1	2
1 "	1.3 "	1	1	1	0
1 "	1.5 "	1	3	1	1
1 "	2.0 "	3	1	2	0

(10) *The immunity conferred by a single injection of a toxin-antitoxin mixture is of long duration.*

Table XII.

Recording the antitoxic value, two years after injection, of guinea-pig *H* injected 3 Nov. 1911, with a mixture containing one test dose of toxin together with between 1.0 and 1.2 units of antitoxin. Large local reaction.

Date	Weight	Weeks after injection	A.T. value per c.c.
22 Jan. 1912	—	11	0.14 unit
1 Feb. 1912	570 grms.	13	0.15 "
3 Feb. 1912	—	13	0.17 "
18 Mar. 1912	565 "	19	0.17 "
21 Mar. 1912	545 "	20	0.15 "
22 May 1912	695 "	29	0.15 "
18 Nov. 1912	935 "	54	0.15 "
20 Aug. 1913	1075 "	94	over 0.09 "

It is important to note from this table that for one year if not for two years, the value maintained a constant level. The variations recorded from the thirteenth to the fifty-fourth week are all within the errors of experiment. This guinea-pig survived an injection of 2.0 c.c. toxin on the occasion of its last test, one year and nine months after its original injection. That the level of concentration of antitoxin in the blood is maintained during such a long period while growth is taking place and in the absence of any further stimulation is remarkable. During the period, the guinea-pig gave birth to eight young and thereby a large amount of the mother's antitoxin was lost. (In several experiments, we found that at birth the antitoxic value per c.c. of the blood of the offspring is the same as that of the mother.) It is extremely probable also the elimination of autogenous antitoxin is continually taking place since we know that antitoxin so closely related as that of the mother is soon got rid of in the offspring. From the increase in weight and consequently of blood content of the animal, it would appear therefore that the total antitoxin present steadily increased for at least a year after the injection.

Table XIII.

Recording the antitoxic value of the blood of guinea-pig *C* injected 6 Sept. 1911 with a mixture containing one test dose of toxin together with 1.2 units of antitoxin. No local reaction.

Date	Weight	Weeks after injection	A.T. value per c.c.
29 Dec. 1911	—	16	0.50 unit
2 Jan. 1912	—	17	0.60 "
5 Feb. 1912	510 grms.	22	0.33 "
18 Mar. 1912	740 "	28	0.21 "
1 April 1912	635 "	30	0.26 "
2 Aug. 1912	650 "	47	0.21 "
18 Nov. 1912	750 "	62	0.11 "
6 Sept. 1913	—	104	0.05 "

Another guinea-pig, S 10. x. 11, injected with a toxin-antitoxin mixture causing local reaction was found to contain 0.15 unit of antitoxin four months after the injection. Tested a year later, the level was found to be the same, and 22 months later the animal tolerated an injection of 2.0 c.c. toxin.

The guinea-pig recorded in Table XIII is given as an exceptional case, in that the immunity produced is high and the subsequent fall rapid.

Rabbits.

(11) *Rabbits injected with a toxin-antitoxin mixture give results similar to those produced in guinea-pigs.*

Rabbits are similar to guinea-pigs in that they possess no normal antitoxin and the m.l.d. is, weight for weight, the same as that for guinea-pigs. Owing to their larger weight, it follows that rabbits can tolerate toxin-antitoxin mixtures fatal to guinea-pigs. Five rabbits were injected with 0.36 c.c. toxin (J 176) and one unit of antitoxin, a mixture just fatal to guinea-pigs. The results are recorded in Table XIV.

Table XIV.

Antitoxic value of serum of rabbits after an injection of a toxin-antitoxin mixture containing one unit of antitoxin and 0.36 c.c. toxin, J 176.

Rabbit	Weeks after injection	A.T. value per c.c.
1	9	·07 unit
2	9	·12 „
.	12	·12 „
3	9	·10 „
11	2	·02 „
.	4	·04 „
.	6	·05 „
.	8	·04 „
12	2	·04 „
.	4	·10 „
.	6	·14 „
.	8	·14 „

All the rabbits recorded in Table XIV were eventually injected with 2.0 c.c. toxin (50–100 times the m.l.d. for a normal rabbit) and in all cases they survived at least five days. Nos. 1 and 11, with the lowest antitoxic value, were severely affected.

Several tests were made upon rabbits 11 and 12 at different intervals, and it will be seen that as in the case of the guinea-pigs (see statement 7) the highest value is not obtained until six or eight weeks after the injection.

(12) *More toxic mixtures produce higher immunity.*

Table XV gives the results obtained in two rabbits injected with a more toxic mixture than that used in the preceding table, *i.e.* 0.38 c.c. toxin + one unit of antitoxin. The degree of immunity produced in both rabbits was higher than that in any rabbit recorded in Table XIV.

Table XV.

Antitoxic value of serum of rabbits after an injection of a toxin-antitoxin mixture containing one unit of antitoxin and 0.38 c.c. toxin, J 176.

Rabbit	Weeks after injection	A.T. value per c.c.
5	8	0.21 unit
.	16	0.21 "
9	12	0.26 "

(13) *Splenectomised rabbits respond to injections of toxin-antitoxin mixtures.*

Two rabbits that had had their spleens removed a few months previously were injected with an L + mixture containing 0.36 c.c. toxin, J 176, and one unit of antitoxin. The results are given in Table XVI.

Table XVI.

Antitoxic value of serum of splenectomised rabbits after an injection of a toxin-antitoxin mixture.

Weeks after injection	Antitoxic value of	
	Rabbit 15	Rabbit 16
0	0.00 unit per c.c.	0.00 unit per c.c.
2	0.01 "	0.01 "
3	0.03 "	0.08 "
4	0.04 "	0.15 "
5	0.04 "	0.16 "
6	0.04 "	0.14 "
7	0.04 "	0.16 "
8	0.03 "	0.12 "
9	0.025 "	0.11 "
10	—	—
11	0.025 "	—
12	—	0.10 "
13	—	0.10 "

Both rabbits were subsequently injected with 2.0 c.c. (50–100 m.l.d.) toxin; rabbit 16 survived and rabbit 15 died three days after the injection. The immunity produced in rabbit 15 was lower than that of any rabbits recorded in Table XIV, and the animal failed to survive a dose of toxin tolerated by all the other rabbits. On the other hand, the immunity produced in rabbit 16 was higher than that of any of the non-splenectomised rabbits. The highest antitoxic value was reached at the fourth or fifth week, which appears to be earlier than is the case with normal rabbits.

Other animals.

(14) *Goats injected with toxin-antitoxin mixtures give results similar to those produced in guinea-pigs.*

Goat No. 12 which was found to contain no normal antitoxin was injected with ten times the L + mixture for a guinea-pig. The dose consisted of 3.8 c.c. of toxin, J 176, together with 10 units of antitoxin. One-tenth of

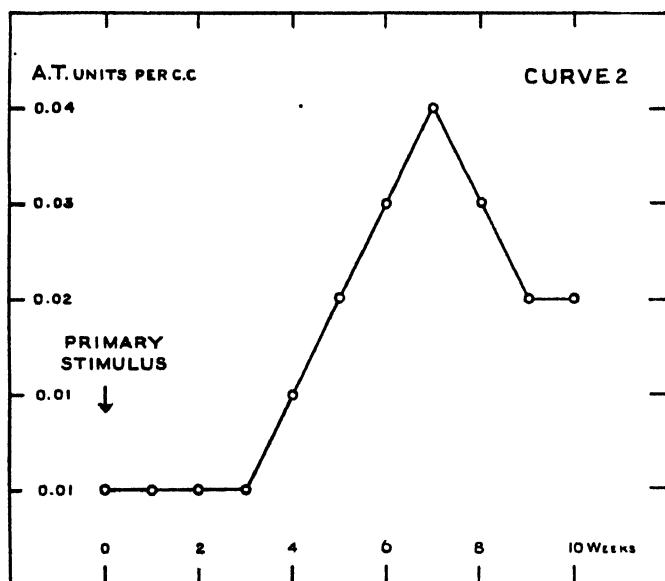
this mixture kills a guinea-pig in 60 hours. The results are recorded in Table XVII.

Table XVII.

Antitoxic value of goat No. 12 after an injection of a toxin-antitoxin mixture.

Weeks after injection	A.T. value per c.c.	Weeks after injection	A.T. value per c.c.
0	0.00 unit	6	0.03 unit
1	0.00 "	7	0.04 "
2	0.00 "	8	0.03 "
3	0.00 "	9	0.02 "
4	0.01 "	10	0.02 "
5	0.02 "		

No antitoxin was detected until the fourth week after the injection, and the highest antitoxic value was recorded at the seventh week. These results are shown graphically in Curve 2.



CURVE 2. From results given in Table XVII, showing the antitoxic value of the serum of a goat possessing no normal antitoxin after an injection of a toxin-antitoxin mixture.

Latent period: 3 weeks. Maximum height: 7 weeks.

- (15) *Horses, possessing no normal antitoxin, injected with toxin-antitoxin mixtures give results similar to those produced in guinea-pigs.*

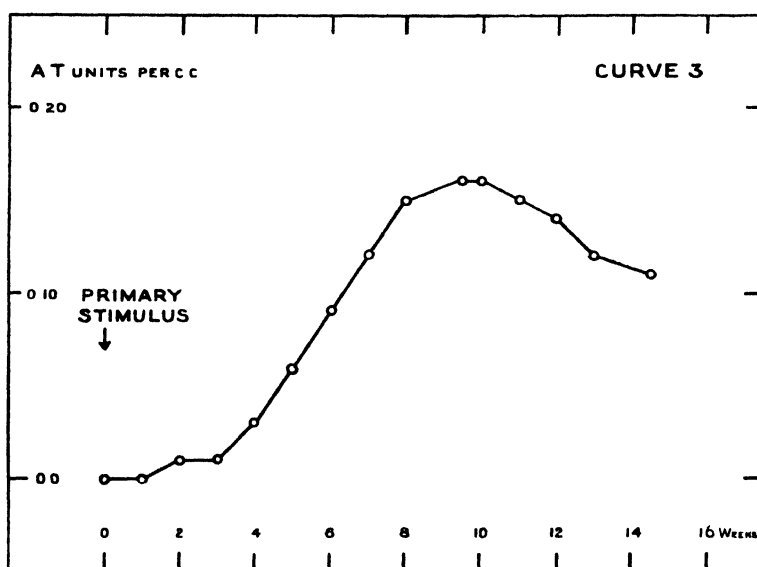
Horse 27 Y, in whose blood no normal antitoxin could be detected, since 1 m.l.d. of toxin mixed with 5 c.c. of its serum killed a guinea-pig, was injected with ten times a toxin-antitoxin mixture that was fatal to guinea-pigs. The dose consisted of 3.6 c.c. of toxin, J 176, together with 10 units of antitoxin. The results are recorded in Table XVIII.

Table XVIII.

Antitoxic value of horse 27 Y after an injection of a toxin-antitoxin mixture.

Weeks after injection	Antitoxic value of serum	Weeks after injection	Antitoxic value of serum
0	0.00 unit per c.c.	8	0.15 unit per c.c.
1	0.00 "	9½	0.16 "
2	0.01 "	10	0.16 "
3	0.01 "	11	0.15 "
4	0.03 "	12	0.14 "
5	0.06 "	13	0.12 "
6	0.09 "	14½	0.11 "
7	0.12 "		

These results are shown graphically in Curve 3.



CURVE 3. From results given in Table XVIII, showing the antitoxic value of the serum of a horse (possessing no normal antitoxin) after an injection of a toxin-antitoxin mixture.

Latent period: 3 weeks. Maximum height: 10 weeks.

(16) *The amount of toxin injected, in relation to the size of the animal, is of importance in the production of immunity.*

A number of other animals that had been found to possess no normal antitoxin were injected with toxin-antitoxin mixtures as follows:

Table XIX.

Animal	Injection	Result
Goat No. 1	1 L + mixture (for a guinea-pig)	No antitoxin detected 9 weeks later
Sheep No. 4	1 "	" " 9 "
2 cows	1 "	" " 4 "
Horse No. 45	1 "	" " 14 "
" No. 46	5 times "	" " 14 "
" No. 47	1 "	" " 3 "

Horses Nos. 45, 46 and 47 were tested weekly.

In all cases the dose employed was relatively small compared with the size of the animal; it is probable that some small amount of antitoxin was produced too small to be detected by the usual tests. That some degree of immunity was produced by these injections is evident from the results of a second injection into the goat, sheep and horse No. 47, recorded later under statements 29 and 31.

That the amount of toxin injected is of importance can be shown by the fact that among guinea-pigs tested for tolerance after an injection of a toxin-antitoxin mixture causing local reaction, we found in the course of our work that those that had received only a fraction of a test dose of toxin showed far less immunity than those receiving a full dose. This point was also brought out in connection with the immunity transmitted to the offspring ("*Immunity of the Guinea-pig to Diphtheria Toxin and its Effects on the Offspring*," *Journal of Hygiene*, vol. XI, No. 2, p. 226).

The importance of the amount of toxin injected may account for the difficulty of immunising with sublethal doses of toxin, and the comparative ease of immunising with toxin rich in toxoid where a number of binding units can be given at a single injection.

(b) ANTITOXIN INJECTED BEFORE TOXIN.

Guinea-pigs.

- (17) *Immunity may be produced by an injection of toxin into guinea-pigs that have previously received heterologous antitoxin; the results are not uniform.*

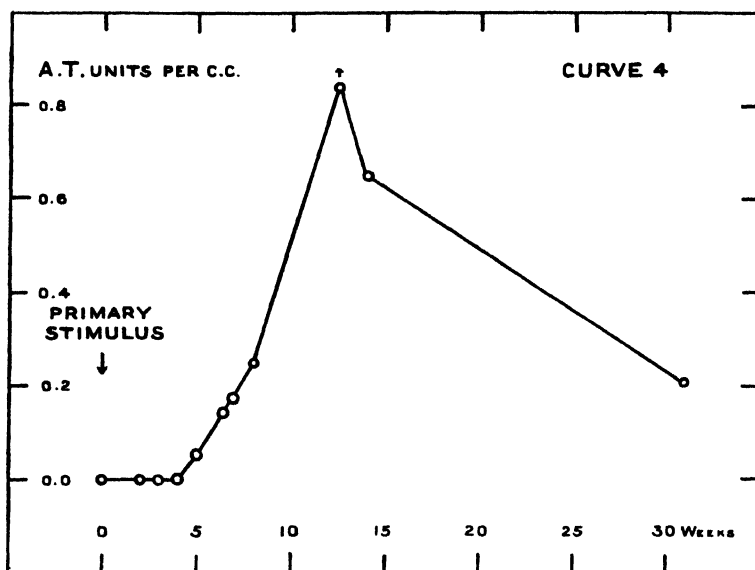
Five guinea-pigs that had been injected with antitoxic horse serum and subsequently injected with toxin were tested for immunity. Immunity was produced in one case only, recorded in Table XX and Curve 4.

Table XX.

Antitoxic value of guinea-pig Z 22. vii. injected with 1000 units diphtheria antitoxic horse serum, and ten days later (when very little antitoxin remained) injected with 0.1 c.c. toxin. Large local reaction, no change in weight.

Weeks after injection of toxin	Weight of guinea-pig	Antitoxic value of blood
2	340 grms.	0.0 unit per c.c.
3	365 "	0.0 "
4	395 "	0.0 "
5	430 "	0.05 "
6½	445 "	0.14 "
7	470 "	0.17 "
8	485 "	0.25 "
12	545 " greater than	0.35 "
12½	575 " "	0.84 "
13	— less than	0.70 "
14	615 "	0.65 "
31	—	0.21 "

As will be seen from Table XX and from Curve 4, no immunity was evident until more than four weeks after the toxin injection, and the antitoxic value continued to rise until the maximum was reached sometime between the eighth and the thirteenth week.



CURVE 4. From results given in Table XX, showing the antitoxic value of the blood of a guinea-pig injected with toxin, ten days after it had received an injection of antitoxin.

Latent period: 4 weeks. Maximum height: 8th-12th week.

In four other cases, no immunity could be detected, due possibly to over-neutralisation of the toxin by the circulating antitoxin, or in some cases to too small a dose of toxin. The various guinea-pigs are compared below.

Table XXI.

Guinea-pig	A.T. value at time of injection	Volume of toxin	Result	Immunity
<i>TT</i> 14. iii. 12	Faint trace	0.01 c.c.	LS + 10	nil
<i>P</i> 22. vii. 12	Under 0.3 unit	0.1 c.c.	LS + 15	nil
<i>Z</i> 22. vii. 12	" 0.5 "	0.1 c.c.	LS 0	Over 0.84 unit per c.c.
<i>Z</i> 30. ix. 12	" 0.7 "	2.0 c.c.	LS - 5	nil
<i>N</i> 14. iii. 12	8.0 "	5.0 c.c.	trace 0	nil

In this method, toxin has been introduced under cover of circulating heterologous antitoxin which is eliminated at a rapid rate; the results are very uncertain. In the next section, the circulating antitoxin is homologous and is not eliminated so rapidly; in this case the results are fairly uniform.

(c) ANTITOXIN PRESENT IN THE FORM OF PASSIVE IMMUNITY TRANSMITTED FROM MOTHER.

Guinea-pigs.

- (18) *A high degree of immunity sometimes results from a single injection of toxin in a guinea-pig passively immune by maternal transmission.*

Ten guinea-pigs that had been bred from actively immune mothers were injected with toxin as soon as they reached a weight of 250 grms., and the antitoxic value of their blood subsequently tested. The results are given in Table XXII. It will be seen that in several instances the antitoxic value of the blood was over $\frac{1}{2}$ unit per c.c., and in one case $1\frac{1}{2}$ units.

It should be noted that the guinea-pigs recorded above were passively immune by maternal transmission and had been slowly eliminating antitoxin since birth. Upon injection of toxin, further loss occurs owing to neutralisation; in no case was any appreciable antitoxin detected two weeks after the injection. If no toxin had been injected, antitoxin would certainly have been present in OO 12.7 A and K 11.7 A for a few weeks later.

The above results are summarised in Table XXIII.

Table XXIII.

Summary of results recorded in Table XXII, of injection of toxin into ten guinea-pigs passively immune by maternal transmission.

Guinea-pig	Vol. of toxin c.c.	Result	A.T. value at time of injection units per c.c. of blood	Highest value reached units per c.c. of blood	Latent period weeks	Interval before highest value reached weeks
A 18.7 D	0.1	trace + 20 grms.	0.04	nil	—	—
OO 12.7 c	0.1	nil + 15 ..	0.26	0.11	$5\frac{1}{2}$	7
C 6.9 B	0.05	VLS 0 ..	0.04	0.21	about $5\frac{1}{2}$	9
OO 12.7 D	1.0	VLS - 20 ..	0.26	0.25	$4\frac{1}{2}$	about 16
A 18.7 c	0.5	VLS + 15 ..	0.04	0.35	$3\frac{1}{2}$ -5	about 14
OO 12.7 A	2.0	LS + 5 ..	1.5	0.38	9	30
C 6.9 A	0.1	SS + 15 ..	0.04	0.46	less than 7	12
N 21.7 c	0.5	VLS + 25 ..	0.07	0.75	$2\frac{1}{2}$ - $3\frac{1}{2}$	8
A 18.7 B	0.1	VLS - 25 ..	0.04	0.97	$2\frac{1}{2}$ - $5\frac{1}{2}$	16
K 11.7 A	8.0	VLS 0 ..	0.92	1.5	2-8	12

The figures given in this table cannot be taken as exact because in several cases the curve was not fully worked out, and some figures depend upon one result only.

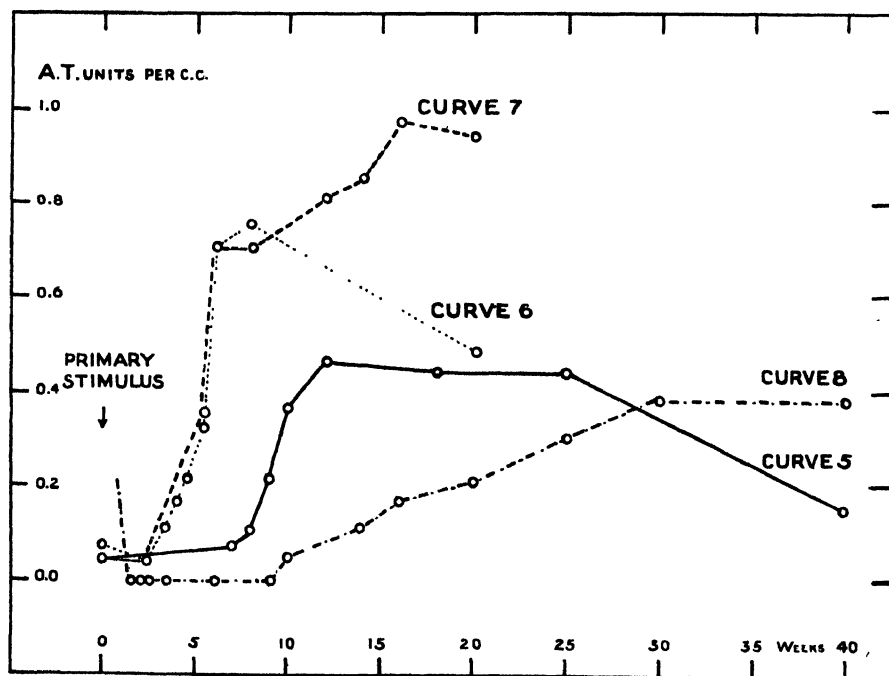
- (19) *The amount of toxin injected must be large in proportion to the antitoxin present in order that immunity may result.*

No definite conclusion can be drawn from Table XXIII, except that the dose of toxin should be high in proportion to the antitoxin present in order that immunity may result. In other words, it would appear that a local reaction must be produced by the toxin. In the first two cases in the table,

no local reaction occurred, and little or no antitoxin resulted. Guinea-pig C 6.9 A appears exceptional in that a high degree of immunity resulted from an injection causing only a small local reaction. In this case, the original antitoxic value of the guinea-pig was low. Two guinea-pigs OO 12.7 c and OO 12.7 d are interesting in that they were from the same litter and injected at the same time when the antitoxic content of the blood was the same. The higher immunity (0.25 unit compared with 0.11) is shown in the guinea-pig which had received the larger toxin dose.

(20) *Comparatively little immunity is produced during the first four weeks after the injection.*

Table XXIII shows the long delay, which may vary from three to nine weeks after the injection, before the appearance of antitoxin. The average latent period appears to be about five weeks. Curves 5 and 6 depicting



Curves 5, 6, 7 and 8. Showing results of injection of toxin into guinea-pigs C 6.9 A, N 21.7 c, A 18.7 B, OO 12.7 A, passively immune by maternal transmission.

	Curve 5	Curve 6	Curve 7	Curve 8
Latent period	7 weeks	2½-3½ weeks	2½-5½ weeks	9 weeks
Maximum height	12th week	8th week	16th week	30 "

C 6.9 A and N 21.7 c respectively are given as typical of this immunity curve. A comparison of these curves with Curves 1-4 will show the resemblance of all immunity curves resulting from a single injection of toxin under cover of antitoxin. Two atypical curves are also given; Curve 7 shows that in the

case of A 18.7 B, the usual rapid rise was followed by a long gradual rise; Curve 8 shows an unusual reaction of guinea-pig OO 12.7 A, from the ninth to the thirtieth week a gradual increase occurred following a prolonged latent period.

- (21) *Summary of Part I. The primary stimulus in guinea-pigs, rabbits, goats and horses is followed by a latent period of about three weeks, and the maximum immunity is reached in about eight weeks.*

Table XXIV summarises the latent period and times of maximum height of immunity for the animals dealt with in Part I. It will be seen that the latent period may be as short as two weeks or as long as nine weeks, but in the majority of instances, it is of three weeks duration. The maximum immunity is reached in one instance in five weeks, usually in from eight to twelve weeks, and in an exceptional instance not until thirty weeks have elapsed.

Table XXIV.

Summary of latent periods and times of maximum height of immunity following the primary stimulus in different animals.

Animal	Nature of stimulus	Reference	Latent period weeks	Maximum height weeks
Guinea-pig	Toxin-antitoxin mixture ...	Curve 1	3	8-12
"	" " ...	Table IX	over 4	over 8
Rabbit	" " ...	" XIV	about 2	" 6
"	" " ...	" XVI	2-3	5
Goat	" " ...	Curve 2	3	7
Horse	" " ...	" 3	3	10
Guinea-pig	Toxin subsequent to antitoxic horse serum ...	" 4	4	8-12
"	Toxin in animals passively immune by maternal transmission ...	" 5	under 7	12
"	" " ...	" 6	$2\frac{1}{2}$ - $3\frac{1}{2}$	8
"	" " ...	" 7	$2\frac{1}{2}$ - $5\frac{1}{2}$	16
"	" " ...	" 8	9	30

PART II.

INJECTION OF TOXIN INTO ACTIVELY IMMUNE ANIMALS

Guinea-pigs.

- (22) *An injection of toxin into an actively immune guinea-pig produces far greater immunity than an injection into a passively immune guinea-pig.*

The highest immunity, recorded in Part I of this paper, produced by an injection of toxin into a passively immune guinea-pig was 1.5 units of anti-toxin per 1 c.c. blood. From subsequent tables it will be seen that an injection of toxin into an actively immune guinea-pig produces far greater immunity; as much as 80 units per c.c. of blood may be produced. Reference

has already been made to a group of guinea-pigs injected with one test dose of toxin together with varying excess of antitoxin, and subsequently injected with 2 c.c. of toxin (see Table X). The antitoxic value of the blood of these guinea-pigs was tested after the injection of toxin; the results are recorded in Table XXV and summarised in Table XXVI.

Table XXVI.

Summary of results of injection of toxin into eight guinea-pigs actively immunised by an injection of a toxin-antitoxin mixture.

Guinea-pig	Primary stimulus		Secondary stimulus		
	Antitoxin in original mixture. Units	Antitoxic value produced by mixture. Unit per c.c. of blood	Result of injection of 2 c.c. toxin	Antitoxic value produced by toxin. Units per c.c. of blood	Approximate time taken to reach highest value
Z 7.11	1.2	0.25	VLS - 5	80	14 days
C 3.7	1.2	0.10	VLS - 55	40-60	over 5 "
T 3.7	1.2	0.20	VLS - 50	28	12 "
K 3.7	1.3	0.11	VLS - 20	16	12 "
B 7.11	1.5	0.09	VLS 0	20-25	about 10 "
X 3.7	1.5	0.25	VLS + 10	8.7	12 "
R 3.7	1.5	0.07	VLS - 30	3.5-6	over 9 "
KK 7.11	1.5	0.07	VLS - 40	0.7	about 14 "

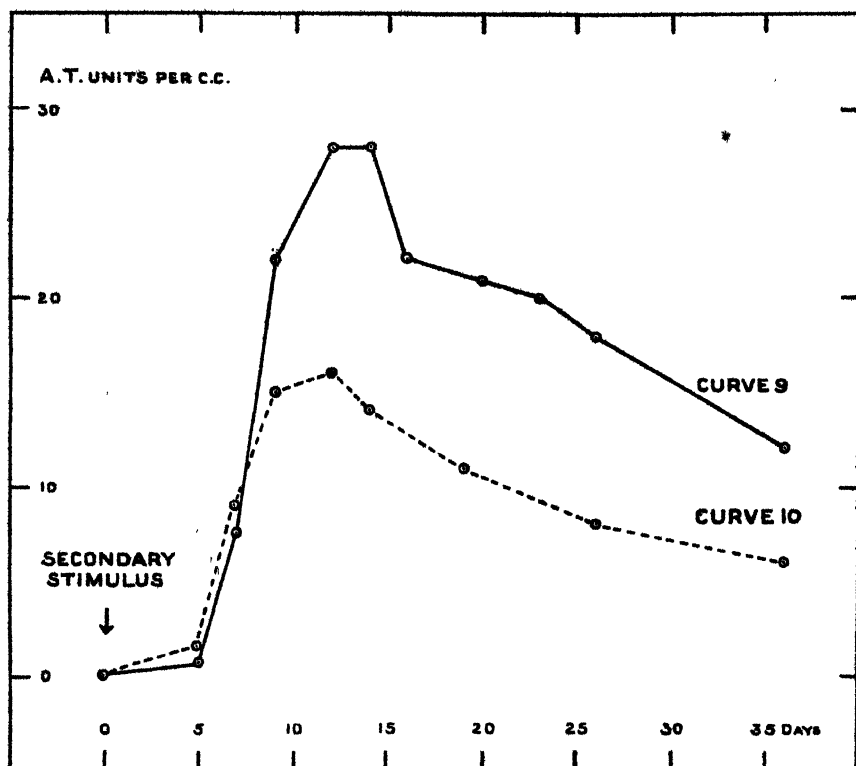
In addition to the animals recorded in these tables, four other guinea-pigs that had received toxin-antitoxin mixtures, causing local reaction, were injected with toxin 12 weeks after the mixture injection. From 20-60 units of antitoxin per c.c. of blood resulted.

(23) *A marked increase of antitoxin occurs within a few days of an injection of toxin into an actively immune guinea-pig and the maximum is reached in about 12 days.*

In passively immune guinea-pigs little or no antitoxin was produced until three or four weeks after injection; eight weeks at least elapsed before the maximum was reached. In the actively immune guinea-pigs recorded in Tables XXV and XXVI an increase in antitoxic value occurred within five days after injection and the maximum was reached about 12 days after. In two cases, where sufficient determinations were made, curves have been plotted. It has been necessary to alter considerably the scale of these curves from that of curves 1-8. The period covered by the earlier curves was 12 weeks or more and in no case was a higher antitoxic value than 1 unit depicted. In the curves here shown a period of only five weeks is covered and an antitoxic value of 28 units recorded.

Curve 9 represents guinea-pig T 3.7 and Curve 10 guinea-pig K 3.7.

The marked difference between the immunity response to the primary and to the secondary stimulus is shown in Curve 11 giving the antitoxic value of guinea-pig K 3.7 during its course of treatment.



CURVES 9 and 10. Injection of toxin into actively immune guinea-pigs *T* 3.7 and *K* 3.7 respectively.

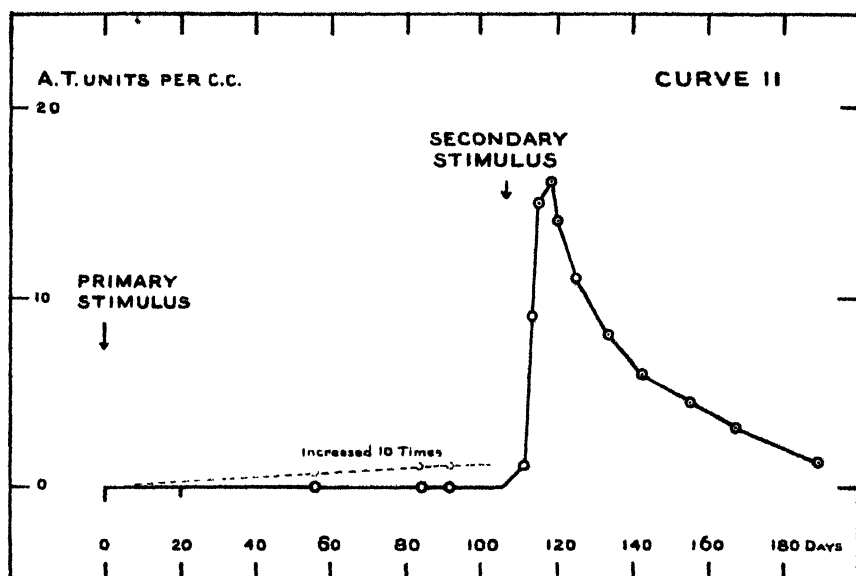
	Curve 9	Curve 10
Latent period	About 5 days	Under 5 days
Maximum height	12 days	12 days

Comparing the two sections of the curve it is seen that the latent period following the injection into the normal animal is approximately five weeks while that following the second injection when the animal is already actively immune is less than five days. The highest value is reached in the one case in 12 weeks and in the other case in 12 days; the highest value after the first injection is 0.11 unit per c.c. and after the second injection 16 units.

(24) *The degree of immunity produced depends partly upon the constitution of the original immunising mixture.*

Neither by tests of antitoxic value nor by the reactions produced by the toxin injection could much distinction be made between the immunity produced in normal guinea-pigs by the injection of toxin-antitoxin mixtures containing 1.2 units of antitoxin and those containing 1.5 units (see Tables X and XI). The antitoxin produced, however, by the injection of 2 c.c. of toxin at least 13 weeks later, differentiates the groups strongly. The average production of antitoxin as a result of the subsequent injection of 2 c.c. of

toxin in the two sets of guinea-pigs, the one of which received a preliminary L0 mixture, while the other received an over-neutralised mixture, is greater in the former than in the latter set.



CURVE 11. Showing the antitoxic value of guinea-pig K 3.7 after an injection of a toxin-antitoxin mixture followed 15 weeks later by an injection of toxin.

In the dotted line after the primary stimulus the ordinates are increased tenfold in order to make apparent the shape of the curve.

	Response to primary stimulus	Response to secondary stimulus
Latent period	Under 8 weeks	Under 5 days
Maximum height	About 12 „	12 days
Maximum unitage	0.11 unit	16 units

The three guinea-pigs originally injected with mixtures containing 1.2 units of antitoxin, produced, as a result of the injection of 2 c.c. of toxin, 28, 40-60, 80 (average 50) units of antitoxin per 1 c.c. of blood. Four guinea-pigs of the other group originally injected with mixtures containing 1.5 units antitoxin eventually produced as a result of the toxin injection 0.7, 4-6, 8.7 and 20-25 (average 10) units of antitoxin.

(25) *Relatively high immunity similarly results from the injection of toxin-antitoxin mixtures into actively immune guinea-pigs.*

High immunity may result from a L0 mixture in place of an injection of toxin in actively immune guinea-pigs. Table XXVII gives the results from injecting L0 mixtures into guinea-pigs that had previously received toxin-antitoxin mixtures.

In Table XXVII the five guinea-pigs are arranged in order of magnitude of immunity response to the secondary stimulus; this order is the same as

that of the time interval elapsing between the primary and the secondary stimulus and of the antitoxic values of the guinea-pigs at the time of the second injection. In Part I it has been shown that the maximum antitoxic value is reached about 12 weeks after the primary stimulus; it would appear therefore that the first two guinea-pigs in the table had already achieved their

Table XXVII.

Results of injecting toxin-antitoxin mixture into five guinea-pigs actively immunised by a previous injection of a toxin-antitoxin mixture.

Guinea-pig	Primary stimulus			Interval weeks	A.T. value at time of second injection	Secondary stimulus		
	Antitoxin in mixture with 1 test dose of toxin	Result				Antitoxin in mixture with 1 test dose of toxin units	Result	Highest value reached units
R 26. 8	less than 1.2 units	LS -21	13	0.60 unit	1.3	nil + 15	20-25	
H 15. 8	more than 1.2 "	nil - 7	13	0.35 "	1.3	nil + 5	20	
K 5. 9	less than 1.2 "	LS -33	11	0.16 "	1.3	nil + 40	1.4	
L 22. 8	1.1 "	VLS - 1	9	less than 0.07 "	1.3	nil + 50	1.0	
O 21. 8	1.3 "	nil - 2	9	" 0.07 "	1.3	nil + 5	0.7	

maximum response to the primary stimulus before the injection of the secondary stimulus. The remaining three animals gave only a poor response to the secondary stimulus received before the maximum effect of the primary stimulus was reached. The conclusion is that an injection given on a rising antitoxin curve is less effective than an injection given when the maximum height has been reached.

(26) *Two injections of a neutral mixture into normal guinea-pigs do not produce any higher immunity than a single injection unless sufficient time elapses between the two injections.*

Three guinea-pigs (of the group marked 7.11 of which Z, B, KK in Table XXV also formed a part) that had received toxin-antitoxin mixtures containing 1.5 units of antitoxin were injected five months later with a similar mixture. These animals produced 0.7, 0.7 and 1.7 units of antitoxin per c.c. of blood. Another set of the same group received a second injection of the mixture seven days after the first injection. Their antitoxic values later were 0.0, 0.11 and 0.14 unit per c.c.; this level is no higher than that reached by animals that received one injection only.

It is of interest here to summarise the results obtained from this group of guinea-pigs.

Table XXVIII.

Summary of results obtained from a single group of guinea-pigs (marked 7.11).

1st injection	Interval	2nd injection	Antitoxin produced	
One LO mixture	—	—	A.T. of the order of 0.1 unit	
LO mixture	7 days	LO mixture	" "	0.1 "
"	Several months	"	" "	1.0 "
"	"	Toxin (causing large local reaction)	" "	10.0 units

From this table it is seen that if a similar L0 mixture is repeated with an interval of one week only, no higher immunity is ultimately reached than when one injection only is given, whereas if an interval of several months elapses between the two injections the resulting immunity is of a much higher order, being ten times the amount in the particular experiment. This shows that a second injection given during the latent period following the first injection acts as a primary and not as a secondary stimulus.

(27) *If a long interval of time (a year or more) elapse before the second injection, high immunity is likewise produced.*

The cases recorded so far were of those with an interval of a few months only between the two injections, that is to say, the second injection was given before there was any appreciable drop in the immunity curve resulting from the first injection. An experiment was made to see whether the same effect would be produced on a dropping curve.

Guinea-pig 223 LA 1. Immunised by a series of injections, in Oct. 1911; 14 units per c.c. resulted. In August 1913 when the antitoxic value had fallen to 0.11 unit per c.c., 2 c.c. of toxin were injected causing very large local reaction; over 20 units A.T. resulted.

A number of other actively immune guinea-pigs were injected but were not individually tested. The pooled serum from these guinea-pigs bled ten days after the injection of toxin contained 30 units per c.c. In all cases at least nine months, and in the majority of cases, over a year had elapsed since the previous injection.

Rabbits.

(28) *The injection of toxin into actively immune rabbits gives results similar to those obtained with guinea-pigs.*

Six of the rabbits previously mentioned (Tables XIV and XV) that had received toxin-antitoxin mixtures were injected 9 to 16 weeks later with 2 c.c. toxin; all developed high immunity within ten days as recorded in Table XXIX.

Table XXIX.

Results of injecting toxin into six rabbits actively immunised by an injection of a toxin-antitoxin mixture.

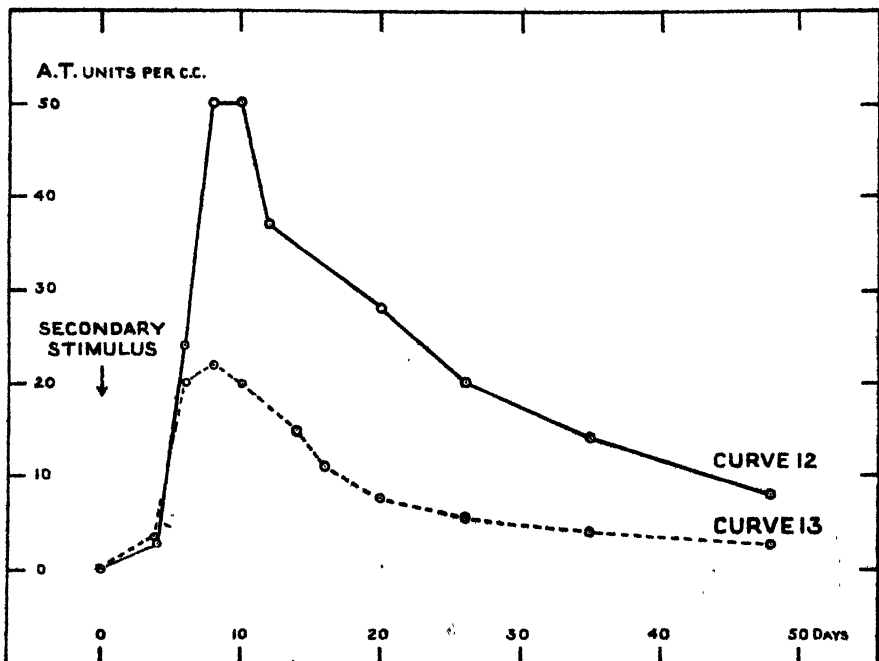
Rabbit	Change in weight 5 days after the injection of 2 c.c. toxin	Maximum antitoxic value reached	Maximum height reached in days after toxin injection
1	- 340 grms.	65 units	10
2	+ 2 "	50 "	8
3	- 130 "	22 "	8
9	- 135 "	28 "	9
11	- 365 "		
12	- 85 "	50 "	10

Table XXX records in detail the results given by two of these rabbits. The antitoxic values are plotted in Curves 12 and 13.

Table XXX.

Results of injecting toxin into two rabbits actively immunised by an injection of a toxin-antitoxin mixture.

Primary stimulus.	Injection of toxin-antitoxin mixture	Rabbit 2	Rabbit 3
Toxin	0.36 c.c.	0.36 c.c.
Antitoxin	1 unit	1 unit
Local reaction...	...	4 × 4 sq. cm.	7 × 6 sq. cm.
Change in weight	...	- 75 grms.	- 110 grms.
Highest A.T. value	...	0.12 unit per c.c.	0.10 unit per c.c.
Secondary stimulus.	Injection of toxin		
Interval between primary and secondary stimulus	...	13 weeks	13 weeks
Volume of toxin injected	...	2 c.c.	2 c.c.
Local reaction...	...	4 × 6 sq. cm.	3 × 3 sq. cm.
Change in weight	...	+ 10 grms.	- 130 grms.
Antitoxin produced			
4 days after toxin injection	...	2.8 units	3.2 units
6	...	24	20
8	...	50	22
10	...	50	20
12	...	37	—
14	...	—	15
16	...	—	11
18	...	—	—
20	...	28	7.5
26	...	20	5.5
35	...	14	4.0
48	...	8	2.8



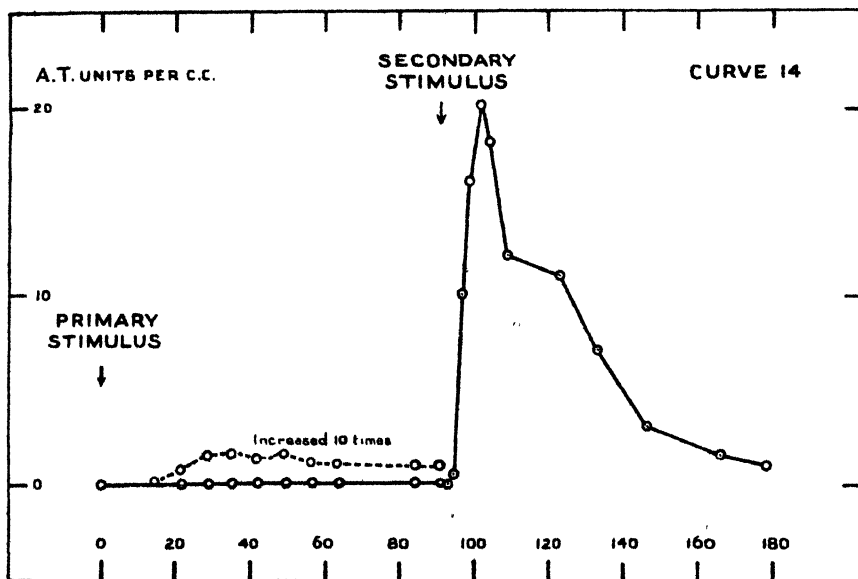
CURVES 12 and 13. Injection of toxin into actively immune rabbits 2 and 3 respectively.

Curve 12 Curve 13
 Latent period Under 4 days Under 4 days
 Maximum height 8 days 8 days

Table XXXI.

Results of injecting toxin into splenectomised rabbit number 16 actively immunised by an injection of a toxin-antitoxin mixture.

Primary stimulus						
Toxin	0.36 c.c.
Antitoxin	1 unit
Highest value	0.16 unit per c.c.
Secondary stimulus						
Interval between primary and secondary stimulus						13 weeks
Volume of toxin injected						2 c.c.
Antitoxic value						
Before injection	0.10 units per c.c.
2 days after	0.04 "
4 "	0.5 "
6 "	10.0 "
8 "	16.0 "
11 "	20 "
13 "	18 "
18 "	12 "
32 "	11 "
42 "	7 "
56 "	3 "
75 "	1.5 "
88 "	1.0 "



CURVE 14. Showing the antitoxic value of a splenectomised rabbit after an injection of a toxin-antitoxin mixture followed 13 weeks later by an injection of toxin.

In the dotted line after the primary stimulus the ordinates are increased tenfold in order to make apparent the shape of the curve.

	Response to primary stimulus	Response to secondary stimulus
Latent period	3 weeks	4 days
Maximum height	5 "	11 "
Maximum unitage	0.16 unit per c.c.	20 units per c.c.

A splenectomised rabbit (number 16) already mentioned in section 13, was injected with 2 c.c. of toxin 13 weeks after the original injection of a toxin-antitoxin mixture. The results are recorded in Table XXXI and Curve 14.

These results conform to those of normal rabbits and clearly show that the spleen plays no essential part in the production of immunity.

The test two days after the secondary stimulus shows the phenomenon of the negative phase.

Sheep and Goats.

(29) *A rapid formation of antitoxin occurs after the injection of toxin into actively immune sheep and goats.*

Goat number 12 recorded in Table XVII was injected with 2 c.c. of toxin ten weeks after the first injection of a toxin-antitoxin mixture. The result of the second injection is recorded in Table XXXII and Curve 15.

Table XXXII.

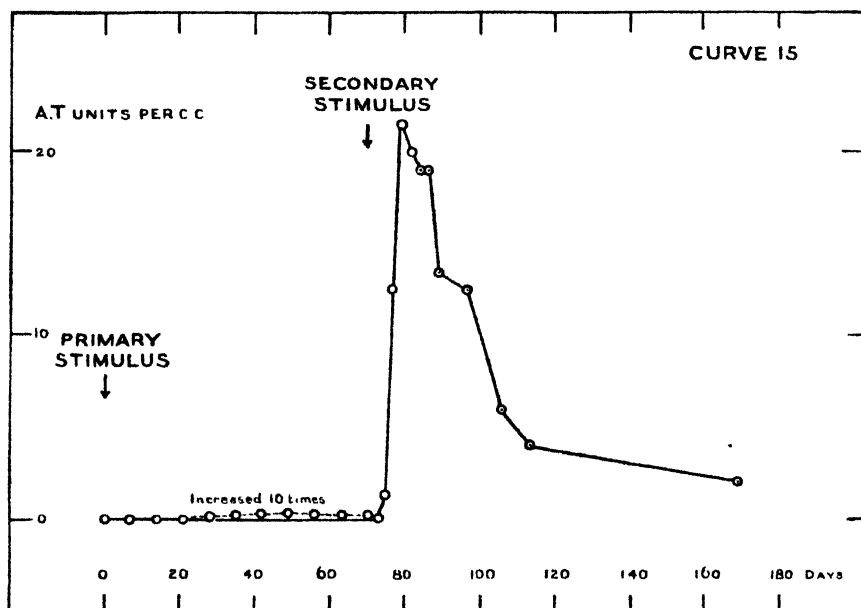
Results of injecting toxin into a goat actively immune from an injection of a toxin-antitoxin mixture.

Primary stimulus						
Toxin	3.8 c.c.
Antitoxin	10 units
Highest value	0.04 unit per c.c.
Secondary stimulus						
Interval between primary and secondary stimulus						10 weeks
Volume of toxin injected						2 c.c.
Antitoxic value						
Before injection	0.02 units per c.c.
3 days after	0.02 "
5 "	1.7 "
7 "	12.5 "
9 "	21.5 "
12 "	20 "
14 "	19 "
16 "	19 "
19 "	13.5 "
27 "	12.5 "
36 "	6 "
43 "	4 "
99 "	2 "
162 "	1 "

The sheep and goat already mentioned in Table XIX were subsequently injected with a second toxin-antitoxin mixture. Tests were made to determine whether a rapid production of antitoxin occurred but the curves were not followed out in detail.

Goat 1. Injected 0.36 c.c. toxin + 1 unit A.T. Nine weeks later no antitoxin detectable in serum. Injected 0.38 c.c. + 1 unit A.T. Nine days later, 0.5 unit. Fourteen days later, 0.65 unit.

Sheep 4. Injected 0.38 c.c. toxin + 1 unit A.T. Nine weeks later no anti-toxin detectable in serum. Injected 0.38 c.c. toxin + 1 unit A.T. Fourteen days later, 0.7 unit. Twenty-one days later, 0.7 unit. Thirty-five days later, 0.15 unit.



CURVE 15. Showing the antitoxic value of a goat after an injection of a toxin-antitoxin mixture followed 10 weeks later by an injection of toxin.

In the dotted line after the primary stimulus the ordinates are increased tenfold in order to make apparent the shape of the curve.

	Response to primary stimulus	Response to secondary stimulus
Latent period	3 weeks	Between 3 and 5 days
Maximum height	7 „	9 days
Maximum unitage	0.04 unit per c.c.	21.5 units per c.c.

In these animals the second injection nine weeks after the first acted as a secondary stimulus; it must be concluded therefore that active immunity resulted from the primary stimulus although the amount of circulating anti-toxin was too small to be detected. The total quantity of toxin injected compared with the size of the animals was very small, being only just above the L + dose for a guinea-pig.

A cow was similarly treated, but only 3½ weeks elapsed between the two injections; no antitoxin was subsequently detected.

Horses.

(30) *The second injection of a toxin-antitoxin mixture causes a rapid production of antitoxin in horses possessing no normal antitoxin.*

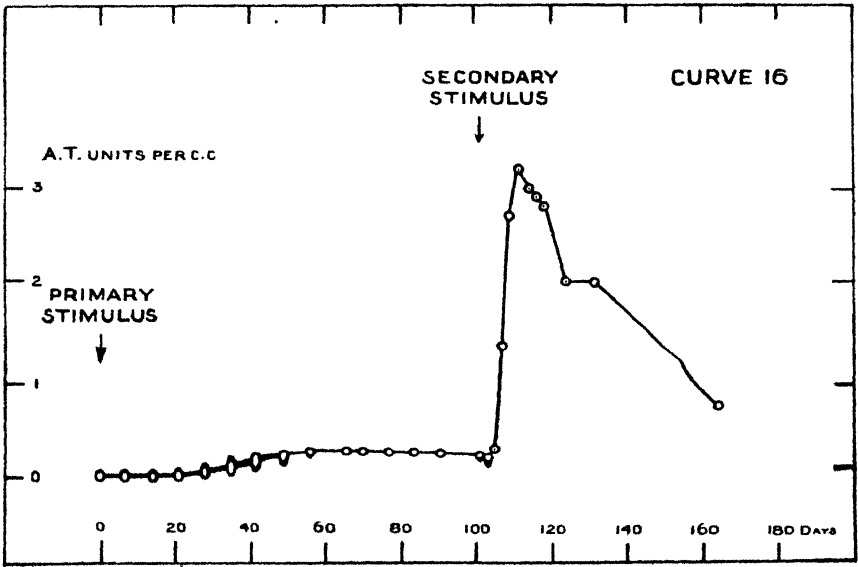
Horse 27 Y previously mentioned in Table XVIII was injected with ten times the L + mixture for a guinea-pig 14½ weeks after the first injection.

A rapid production of antitoxin occurred as recorded in Table XXXIII and Curve 16.

Table XXXIII.

Results of injection of a toxin-antitoxin mixture into a horse actively immunised by a previous injection of a toxin-antitoxin mixture.

Primary stimulus						
Toxin	3.6 c.c.
Antitoxin	10 units
Antitoxin produced	0.16 unit per c.c.
Secondary stimulus						
Interval between primary and secondary stimulus						14½ weeks
Toxin	3.6 c.c.
Antitoxin	10 units
Antitoxin produced						
Before second injection	0.11 units per c.c.
2 days after	0.10 "
4 "	0.2 "
6 "	1.3 "
8 "	2.7 "
10 "	3.2 "
13 "	3.0 "
15 "	2.9 "
17 "	2.8 "
23 "	2.0 "
31 "	2.0 "
63 "	0.75 "



CURVE 16. Showing the antitoxic value of a horse, originally possessing no normal antitoxin, after an injection of a toxin-antitoxin mixture, followed 14½ weeks later by a similar mixture

	Response to Primary stimulus	Response to secondary stimulus
Latent period	3 weeks	Between 2 and 4 days
Maximum height	9½ "	10 days
Maximum unitage	0.16 unit per c.c.	3.2 units per c.c.

The curve for this horse is particularly interesting because both primary and secondary stimulus consisted of toxin-antitoxin mixtures of the same constitution.

(31) *The response to a second injection rapidly following the primary stimulus in a horse possessing no normal antitoxin is of an intermediate character between the usual responses following the primary and the secondary stimulus.*

A horse with no normal antitoxin (*H 47*) previously mentioned in Table XIX, was injected with a toxin-antitoxin mixture containing five L + doses for a guinea-pig three weeks after the initial injection of a mixture containing one L + dose. The antitoxin response is recorded in Table XXXIV and Curve 17.

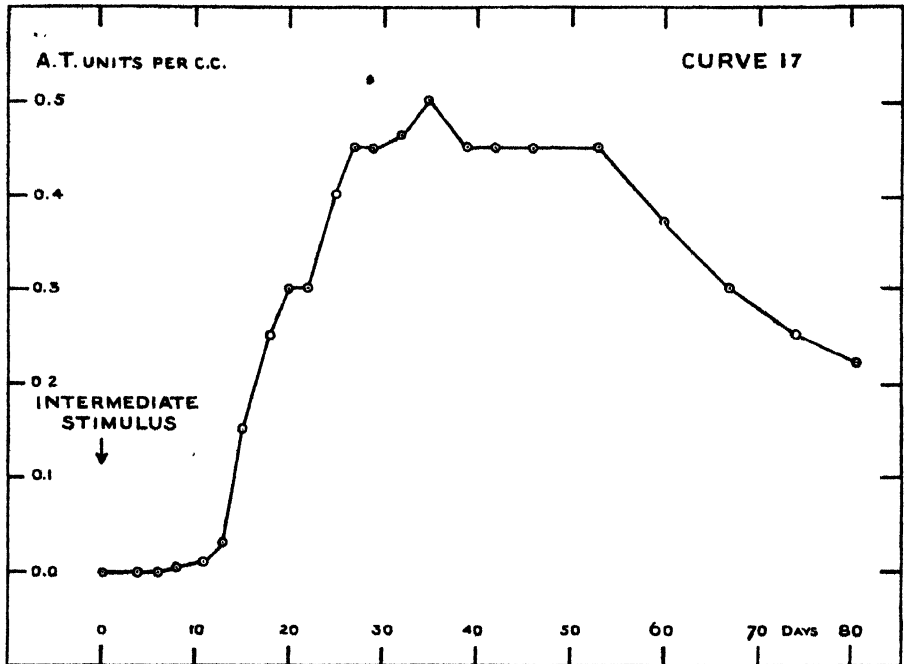
Table XXXIV.

Results of injection of a toxin-antitoxin mixture into a horse three weeks after the initial injection of a similar mixture.

Primary stimulus							
Toxin	0.36 c.c.
Antitoxin	1 unit
Antitoxin produced	Not detectable
Intermediate stimulus							
Interval between primary and intermediate stimulus							3 weeks
Toxin	1.8 c.c.
Antitoxin	5 units
Antitoxin produced							
1 days after second injection	0.00 unit per c.c.
6	"	"	0.00 "
8	"	"	0.005 "
11	"	"	0.01 "
13	"	"	0.03 "
15	"	"	0.15 "
18	"	"	0.25 "
20	"	"	0.3 "
22	"	"	0.3 "
25	"	"	0.4 "
27	"	"	0.45 "
29	"	"	0.45 "
32	"	"	0.46 "
35	"	"	0.50 "
39	"	"	0.15 "
42	"	"	0.45 "
46	"	"	0.45 "
53	"	"	0.45 "
60	"	"	0.37 "
67	"	"	0.30 "
74	"	"	0.25 "
81	"	"	0.22 "

The second injection in this horse was given when the animal was partially immunised and can be termed an intermediate stimulus. The latent period of eight days lies between the usual three weeks for a primary stimulus and

three days for secondary stimulus; similarly the maximum height was reached in 35 days, a period intermediate between ten days and eight weeks.



CURVE 17. Showing the antitoxic value of a horse, originally possessing no normal antitoxin, after a second injection of a toxin-antitoxin mixture three weeks after the first injection.

Latent period: 8 days. Maximum height: 35 days.

(32) *Summary of Part II. The secondary stimulus in guinea-pigs, rabbits, goats, sheep and horses is followed by a latent period of about four days and the maximum immunity is reached in about ten days.*

Table XXXV summarises the latent period and times of maximum height of immunity for the animals dealt with in Part II. It will be seen that after a secondary stimulus the latent period is never more than five days and the maximum height of immunity is reached in from eight to twelve days.

Table XXXV.

Summary of latent periods and times of maximum heights of immunity following the secondary stimulus in different animals.

Animal	Nature of stimulus	Reference	Latent period	Maximum height
Guinea-pig	Toxin	Curve 9	about 5 days	12 days
"	"	" 10	under 5 "	12 "
Rabbit	"	" 12	" 4 "	8 "
"	"	" 13	" 4 "	8 "
"	"	" 14	4 days	11 "
Goat	"	" 15	3 to 5 days	9 "
Horse	Toxin-antitoxin mixture	" 16	2 to 4 "	10 "

PART III.

INJECTION OF TOXIN INTO ANIMALS POSSESSING
NORMAL ANTITOXIN.

Horses.

(33) *The injection of toxin into horses possessing normal antitoxin produces a rapid increase in antitoxin.*

The effect of a single injection of toxin into a horse (*H 32 v*) naturally immune is recorded in Table XXXVI and Curve 18.

Table XXXVI.

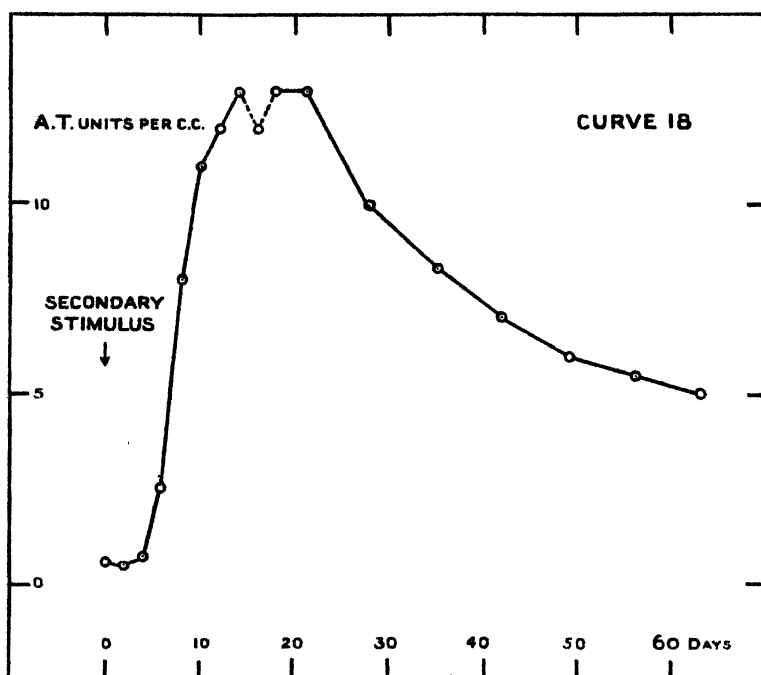
Results of injecting toxin into a horse naturally immune.

Primary stimulus. Probable infection in normal life						
Antitoxin produced	0.6 unit per c.c.
Secondary stimulus						
Interval between primary and secondary stimulus	Unknown					
Toxin	10 c.c.
Local reaction	20 sq. in. lasting 3 days
Temperature reaction	Nil
Antitoxin produced						
2 days after injection	0.5 units per c.c.
4 "	"	"	0.65 "
6 "	"	"	2.5 "
8 "	"	"	8 "
10 "	"	"	11 "
12 "	"	"	12 "
14 "	"	"	13 "
16 "	"	"	12 "
18 "	"	"	13 "
21 "	"	"	13 "
28 "	"	"	10 "
35 "	"	"	8.3 "
42 "	"	"	7 "
49 "	"	"	6 "
56 "	"	"	5.5 "
63 "	"	"	5 "

The similarity of the curve of antitoxin produced in this horse to that obtained after a secondary stimulus in an animal actively immunised by a primary stimulus is strongly suggestive evidence that the antitoxin found in normal horses has been formed by a process of active immunisation.

A number of horses with varying normal antitoxin content were injected with 1 c.c. of weak toxin (*Y 29*: m.l.d. = 0.04 c.c.) and their blood tested nine or ten days later. In one case 20 units of antitoxin per c.c. of serum were produced after this single injection of less than one L + dose for a guinea-pig. The total serum content of a horse may be taken as 25 litres; 500,000 units were therefore produced, in other words a single injection of toxin stimulated the production of as much antitoxin as would neutralise

500,000 times the amount of toxin injected. The summary of results given in Table XXXVII shows that the majority of horses had developed considerable immunity within ten days after the injection.



CURVE 18. Showing the antitoxic value after an injection of toxin into a naturally immune horse.
Latent period: 4 days. Maximum height: 14 days.

Table XXXVII.

Results of injecting toxin into 103 horses possessing normal antitoxin.

Increase of antitoxic value within ten days	Number of horses
Less than 0.5 unit per c.c.	23
Between 0.5 and 1.0 unit per c.c.	7
„ 1.0 „ 5.0 units per c.c.	55
Over 5.0 units per c.c.	18

A group of horses injected with 1 c.c. of the same weak toxin were examined more closely and are recorded in Table XXXVIII.

It is of interest to note that in four cases a fall in antitoxic value was recorded in the first three days after injection. No such alteration in normal antitoxic content has yet been noted during such a short time in untreated horses. The quantity of toxin injected (under one test dose) would, by neutralisation, only reduce the antitoxic value by $1/25,000$ unit and this reduction would be undetected. It follows therefore that the phenomenon cannot be due only to neutralisation by the toxin, but must be regarded as an instance of the negative phase.

The above results are shown graphically in Curves 19-23.

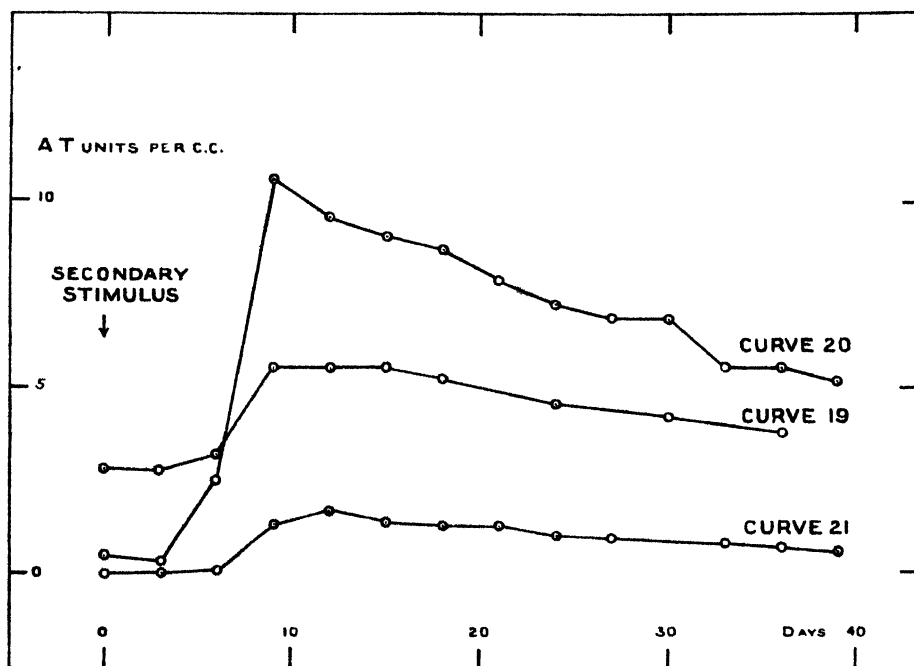
Table XXXVIII.

Results of injecting toxin into five horses possessing normal antitoxin.

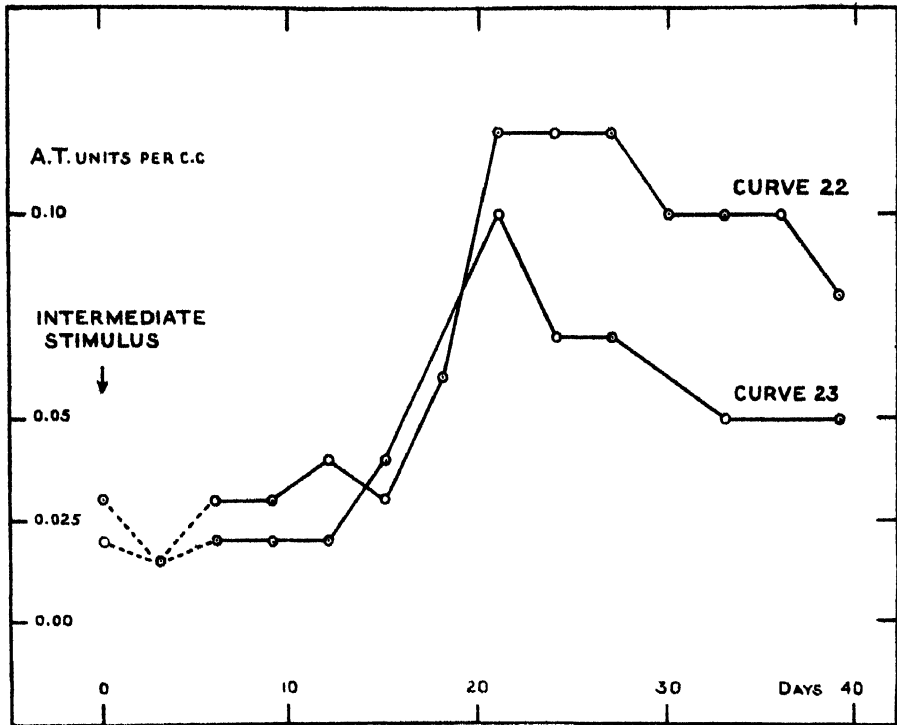
	Horse 6 <i>N</i>	Horse 56 <i>C</i>	Horse 27 <i>S</i>	Horse 69	Horse 63
Normal antitoxic value in units per c.c. ...	2.8	0.5	0.05	0.05*	0.02
Volume of toxin injected	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Local reaction ...	4 sq. in.	6 sq. in.	32 sq. in.	192 sq. in.	225 sq. in.
Duration of local reaction	2 days	2 days	5 days	7 days	9 days
Rise in temperature ...	nil	nil	1° F.	1.8° F.	3.4° F.

Days after injection	A.T. value produced in units per c.c.					
	2.75	0.35	0.03	under 0.02	under 0.02	0.02
3						
6	3.2	2.5	0.08		0.03	0.02
9	5.5	10.5	1.3		0.03	0.02
12	5.5	9.5	1.7		0.04	0.02
15	5.5	9.0	1.4		0.03	0.04
18	5.2	8.7	1.3		0.06	0.07
21	—	7.8	1.3		0.12	0.10
24	4.5	7.2	1.0		0.12	0.07
27	—	6.8	0.9		0.12	0.07
30	4.2	6.8	—		0.10	—
33	—	5.5	0.8		0.10	0.05
36	3.75	5.5	0.7		0.10	—
39	—	5.2	0.6		0.08	0.05
42	3.2	4.7	0.5		0.08	—

* Probably an over-estimate—strong evidence in favour of normal value being 0.03 unit per c.c.

CURVES 19, 20, 21. Showing the antitoxic value of three naturally immune horses 6 *N*, 56 *C* and 27 *S* after an injection of toxin.

	Curve 19	Curve 20	Curve 21
Latent period	3-6 days	3-6 days	3-6 days
Maximum height	9 "	9 "	12 "



CURVES 22, 23. Showing the antitoxic value of two naturally immune horses 69 and 63 after an injection of toxin.

	Curve 22	Curve 23
Latent period	15-18 days	15 days
Maximum height	21 "	21 "

(34) *The injection of toxin into horses possessing very little immunity acts as an intermediate stimulus.*

The first two horses, 6 *N* and 56 *C*, recorded in Table XXXVIII and Curves 19 and 20, showed by their normal values and by the small size and short duration of their local reactions produced by the toxin that they possessed a high degree of natural immunity. Horse 27 *S*, recorded in Table XXXVIII and Curve 21, had a relatively small antitoxic content, but the small local reaction produced by the toxin showed that the horse possessed a considerable degree of immunity. The injection of toxin into these three horses gave rise to the typical secondary stimulus phenomenon. On the other hand, the last two horses, 69 and 63, recorded in Table XXXVIII and Curves 22 and 23, showed a low normal antitoxin content, and both gave very large local reactions to the toxin. The long latent period and delay in reaching the maximum height are typical of the intermediate stimulus phenomenon.

- (35) *A toxin-antitoxin mixture may produce considerable immunity in a naturally immune horse.*

Horse 38 *H*, recorded in Table XXXIX, was injected with a toxin-antitoxin mixture containing five L + doses for a guinea-pig.

Table XXXIX.

Primary stimulus. Probable infection in normal life							
Antitoxin produced		0.45 unit per c.c.
Secondary stimulus							
Interval between primary and secondary stimulus							Unknown
Toxin	1.9 c.c.
Antitoxin	5 units
Antitoxin produced							
3 days after injection			0.8 units per c.c.
6	”	”	1.5 ”
9	”	”	6.0 ”
12	”	”	6.0 ”
15	”	”	6.0 ”
18	”	”	5.0 ”

Humans.

- (36) *The injection of a toxin-antitoxin mixture into a naturally immune human causes a rapid production of antitoxin.*

Table XL records the results of injecting a toxin-antitoxin mixture containing three L0 doses for a guinea-pig into a naturally immune human (A. T. G.).

Table XL.

Primary stimulus. Probable infection in normal life							0.10 unit per c.c.
Antitoxin produced		
Secondary stimulus							Unknown
Interval between primary and secondary stimulus							
Toxin	3 L0 doses
Antitoxin	3 units
Antitoxin produced							0.16 unit per c.c.
2 days after injection			
4	"	"	0.20 "
7	"	"	0.5 "
15	"	"	1.0 "
21	"	"	1.0 "

- (37) *Summary of Part III. The injection of toxin into a naturally immune horse and human acts as a secondary stimulus and is followed by a latent period of about four days, and the maximum height of immunity is reached in about ten days.*

Table XLI summarises the latent periods and times of maximum height of immunity for the animals dealt with in Part III. The periods of time recorded correspond closely with those given in Table XXXV.

Table XLI.

Summary of latent periods and times of maximum height of immunity following the secondary stimulus in naturally immune animals.

Animal	Nature of stimulus	Reference	Latent period		Maximum height
			4 days		14 days
Horse	Toxin	Curve 18			
"	"	" 19	3-6	"	9 "
"	"	" 20	3-6	"	9 "
"	"	" 21	3-6	"	12 "
"	Toxin-antitoxin mixture	Table XXXIX	under 3	"	9 "
Human	" "	" XL	" 2	"	7-15 "

(38) *The injection of toxin into a "partially" immune animal acts as an intermediate stimulus.*

Three instances have been recorded, one in Part II of an artificially immunised horse, and two in Part III of naturally immune horses in which the immunity response was delayed. To this delayed response, we have applied the term "Intermediate Stimulus Phenomenon." A summary of the three horses is given in Table XLII.

Table XLII.

Animal	Nature of stimulus	Reference	Latent period	Maximum height
Horse	Toxin-antitoxin mixture	Curve 17	8 days	35 days
"	Toxin	" 22	15-18 "	21 "
"	"	" 23	15 "	21 "

SUMMARY.

(a) *Primary Stimulus.* In animals possessing no normal antitoxin a single injection of toxin either "attenuated" or under cover of antitoxin, whether injected previously or at the same time or present in the form of passive immunity maternally transmitted, is followed by a latent period of about three weeks, and the maximum immunity is reached in about eight weeks.

(b) *Secondary Stimulus.* In immune animals, whether naturally immune or artificially immunised, a single injection of toxin or of a toxin-antitoxin mixture is followed by a latent period of about four days and the maximum immunity is reached in about ten days; the great and rapid immunity response to the secondary stimulus offers a striking contrast to the small and gradual response to the primary stimulus.

(c) *Intermediate Stimulus.* In partially immune animals the response to an injection of toxin is in magnitude and rapidity of a character intermediate between the responses following a primary and a secondary stimulus.

A NEW METHOD OF OBTAINING CULTURES FROM SINGLE BACTERIAL CELLS.

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(With Plate I.)

WHILE investigating variations in agglutinability, occurring among certain strains of paratyphoid bacilli, it became desirable to obtain cultures derived from single bacterial cells. The technique at first adopted was that devised by Mutch (1919), but the results obtained were not satisfactory. Certain modifications of this method were attempted, but were no more successful.

The main difficulty, and one which appears to us to have been far too little emphasised in all the methods hitherto described, has been encountered in obtaining satisfactory conditions for the microscopical observation of the bacillary suspensions employed. The difficulty of accurately observing hanging-drop preparations, and still more of controlling manipulation of them by microscopical observation, would seem to us to have been insufficiently realised; and the ease with which faulty interpretation of microscopical appearances may introduce fatal errors into work of this kind has not, perhaps, been fully taken into account.

For this reason we first turned our attention to the problem of securing really satisfactory conditions for microscopical observation, and adopted the dark-ground method of illumination for this purpose. Using this technique, with a relatively granule-free medium for suspension, and examining thin films between a slide and a cover-slip, it is possible to pick out a single bacterial cell, lying apart from any other organism, with a certainty quite unobtainable in the observation of hanging-drop preparations by directly transmitted light, or of organisms situated on the surface of any solid nutrient medium. The conditions of this method of observation, on the other hand, entirely preclude subsequent isolation of an individual bacterial cell by mechanical means, while the experience met with in attempts to examine large series of such preparations, until one was found which contained a single bacterium only, did not offer any inducement to continue work along these lines.

For these reasons we definitely abandoned all attempts to obtain the desired results by methods such as those described by Barber (1908, 1911, 1914, 1920), Mutch (1919), Hort (1920), Hewlett (1918), Malone (1918), and others; and tried to discover a solution of the difficulty along entirely new

lines. The vital factor of good optical conditions for the examination of the preparations appeared to us to be satisfactorily dealt with by the use of thin film-preparations of young bacterial growths in granule-free nutrient gelatine, using dark-ground illumination. Such preparations overcome the difficulty introduced by the motility of certain organisms.

Previous investigations by one of us (Barnard and Morgan, 1903 *a* and 1903 *b*) suggested the possibility of utilising the sterilising action of ultra-violet light to kill all bacteria in the preparation, with the exception of the single cell from which growth was to be obtained. The problem remained of devising a method by which this cell alone might be protected. The solution was found in covering the organism selected with a small mercury globule, between $50\ \mu$ and $120\ \mu$ in diameter. Such small globules cling to the surface of the cover-slip so tenaciously that there is no fear of displacing them during the subsequent manipulations, if reasonable care be exercised.

THE TECHNIQUE EMPLOYED.

The only apparatus required, in addition to that which will be found in any bacteriological laboratory, is a mercury arc as the source of ultra-violet radiation, and a supply of circular quartz cover-slips of about 12 mm. diameter. The actual procedure is as follows.

A culture of the bacterial strain, from which the isolation is to be made, is put up in ordinary nutrient broth and allowed to grow for about 18 hours at 23°C ., or about 6 hours at 37°C . A tube of 10 per cent. gelatine in 1 per cent. peptone-water is then melted, cooled to 37°C . and inoculated with a loopful of the actively growing broth-culture. This is then replaced in the incubator at 37°C . for another two hours. These times are of course only approximate, but if the most satisfactory results are to be obtained they must not be widely departed from. It is of great importance to work with young, actively growing cultures, containing relatively few bacteria, the great majority of which are viable.

Several (6-12) preparations are now put up by placing a small drop of the liquid gelatine culture on a quartz cover-slip, using a platinum loop with an external diameter of 3 mm., and inverting this on a glass slide, both slide and cover-slip having been previously sterilised by passing them through the flame of a Bunsen burner and allowing them to cool under a glass cover. The slide must be of a suitable thickness for use with a dark-ground condenser (0.95-1.1 mm.). The drop of culture should be of such a size that a very thin film is formed, completely filling the space between the slide and the cover-slip without any escape beyond the edge of the latter. This film should be entirely free from air-bubbles.

• These preparations are then examined, using a $1/6$ objective and an 18 compensating ocular. A suitable preparation will show 5 to 20 bacteria in the whole film, any one cell being widely separated from its nearest neighbour. In choosing a cell for isolation certain facts should be kept in mind.

It is important to select an organism which is surrounded by a wide zone of medium, in which careful examination fails to reveal any other bacteria. It is best to choose a cell which already shows signs of incipient division, and further manipulations are facilitated by selecting an organism which lies near the centre of the preparation, rather than one near the periphery.

The choice having been made the cell selected is moved to the centre of the field, and its position is read off on the verniers of the mechanical stage. (Fig. 1 shows such a cell in position.) The $1/6$ objective is now replaced by a $2/3$, and the cell is again identified.

A large drop of carefully cleaned mercury is now placed in a glass dish and broken up into minute droplets by a smart tap with the end of a finger. One of these minute droplets is picked up on the point of a needle and transferred to the surface of the cover-slip, at a point as close as possible to the centre of the field of illumination. For this procedure it is best to employ a needle with a roughened point which has been allowed to rust. The mercury droplet will often refuse to cling to a clean and polished needle. The exact position of the mercury droplet is then ascertained by examination with the $2/3$ objective and 18 ocular. The appearance presented when the edge of the droplet is focussed is shown in Pl. I, Fig. 2. It has now to be moved into position, so as to lie with its centre over the cell to be protected. It is simply pushed into its proper place with the point of an ordinary steel needle, under observation through the microscope. This procedure is singularly easy after a little practice. The needle is held at a slight angle with the horizontal, and the point only should be used. The manipulation is greatly facilitated by the fact that the reflection of the point of the needle is clearly observable on the surface of the mercury droplet, long before the point itself appears in the field. To make use of this fact the tube of the microscope is racked up slowly, so that successive bright rings, which are reflections from the upper convex surface of the mercury droplet, come into view. The appearance of such a drop is shown in Fig. 3. At the upper part, between the inner and outer rings is seen a point of light, and below this there is a small break in the inner illuminated ring. This appearance is the reflection of the needle point. The needle employed at this stage should be scrupulously clean and polished, since a dirty or roughened point may cause the droplet to cling to it and be dragged back when the needle is withdrawn. The droplet having been successfully manœuvred into the centre of the field, the $2/3$ is again replaced by the $1/6$ objective. The drop should now be found to fill at least half the field, but must not fill it completely. On racking down the tube of the microscope the bacterial cell may now be observed in the centre of the field, below the centre of the mercury droplet. The possibility of thus controlling the position of the droplet in relation to the bacillus, by direct observation, depends upon the fact that the size of the former is very small, relatively to the aperture of the objective. On lowering the tube of the microscope the mercury droplet passes out of focus and disappears from view, while the

rays entering the objective peripherally form an image of the bacterium, which is readily observable, though less bright than that obtained without the interposition of the droplet. If the droplet employed be so large as almost to fill the microscopic field, a central dark area will be left when the tube is lowered; so that, if the bacillus be lying beneath the centre of the droplet, it will not come into view. This very fact may, however, be taken as evidence that it is satisfactorily covered. The slide is now removed from the stage of the microscope, and the process is repeated with two or three similar preparations. At least two further preparations, without mercury droplets, are reserved as controls.

All these preparations are now exposed to ultra-violet radiation. Using the medium referred to above, we have found that an exposure of one minute, at a distance of three inches from the source of light, gives entirely satisfactory results with such non-sporing organisms as we have studied. The exposure is made through a tube of about 24 mm. diameter, to prevent the incidence of very oblique rays, which might pass beneath the edge of the droplet and reach the underlying cell.

Each preparation is now ringed round with melted paraffin, delivered from a capillary pipette. It is important that this manipulation should be carried out rapidly, and that the temperature of the paraffin should not be far above its melting point. If these precautions be observed there is no danger of melting the gelatine, which is the accident to be avoided.

All preparations are then incubated over night at 25° C. Next morning they are examined with the 1/6 objective and 18 ocular. Such examination should show that the single bacterial cell has now multiplied and formed a colony, containing few or many bacteria (Fig. 4), while any other bacteria in the preparation have failed to divide. These latter will usually present a definitely granular and degenerate appearance. Each of those preparations in which such a colony has formed, and the two or more controls which were exposed without the protection of the mercury droplets, are now subcultured by removing the cover-slip, the edge of the cover-slip and the surrounding surface of the slide being previously sterilised with a hot metal rod. The gelatine is then rubbed up in a loopful of broth, and this is transferred to a tube containing a suitable culture medium. Subsequent examination of these tubes should show growth in some or all of those inoculated from the microscopic colonies, while the controls should remain sterile. These controls are added to demonstrate that the action of the ultra-violet light has not been merely to prevent the multiplication of the unprotected bacteria in the gelatine preparation, but has actually killed them. In our experiments large numbers of such controls have been employed to test the adequacy of the technique. In routine isolation they are not, perhaps, strictly necessary, once the length of exposure needed in the case of the organism and medium to be worked with has been determined; but it is always more satisfactory to include at least two such preparations, and to discard any strains which have been

isolated in an experiment, in which growth has occurred from the unprotected controls. The risk of air-borne contamination of the preparations during the necessary manipulations appears to be negligible, for in our experience it has never occurred.

A few further technical points may be mentioned. The constitution of the medium used in the actual isolation has a profound effect on the length of exposure needed to kill the unprotected bacteria. In many of our earlier experiments the gelatine medium employed was prepared with a tryptic digest of casein, containing large amounts of tyrosin and other amino-acids. Using this medium, the lethal dose of radiation at a distance of three inches lay between four and six minutes, as compared with the one minute required with peptone-gelatine. The mercury arc generates an appreciable amount of heat; and it is, of course, essential that no liquefaction of the gelatine should occur during the exposure. With 10 per cent. gelatine there is little risk of this, except in very hot weather. Under these conditions the risk can readily be obviated by laying the slide on a block of ice while exposing it to the radiations.

With organisms which themselves produce liquefaction of the gelatine, the formation of circumscribed colonies cannot be observed. Since, however, no liquefaction occurs during the time of manipulation and exposure, the technique is readily applicable to such bacteria, and the sterility on subculture of the unprotected controls affords satisfactory evidence of the lethal action of the radiations.

The slides and cover-slips, after subcultures have been made from the gelatine preparations, are most readily sterilised and cleaned by immediately transferring them to 25 per cent. sulphuric acid containing 10 per cent. of potassium bichromate, and boiling them for a few minutes.

As regards the proportion of successful isolations, it is only in connection with the members of the paratyphoid group that our figures are sufficiently large to give representative results. After the main lines of the technique had been arrived at, but while we were still making small variations in the composition of the medium and the length of exposure, we attempted 148 isolations of organisms of this type. In 58 cases (39 per cent.) microscopic colonies were obtained from the single cells selected. In 39 instances (26 per cent.) these microscopic colonies were successfully subcultured. In the last 46 isolations, when the technique described above was strictly adhered to, the percentage of colony-formation was 41 and of successful subculture 33. During the 148 isolations referred to 137 unprotected controls were exposed. Of these 128 remained sterile. The 9 which grew were included in three experiments, in two of which a considerable interval had been allowed to elapse between putting up the preparations used for isolation and those used as controls. During this interval multiplication had been going steadily forward, so that the control preparations probably contained far too large a number of bacteria. Actually, therefore, the number of isolations which have to be discarded through non-sterility of the unprotected controls is very small indeed.

As a further proof of the adequacy of the method it may be mentioned that we have, on many occasions, worked with a mixed culture of *B. coli* and paratyphoid bacilli, or with a mixture of different serological types of the latter group, and have in every case demonstrated the purity of the cultures finally obtained.

Although 33 per cent. of successful isolations may not appear an entirely satisfactory result, it is in practice quite adequate for the purpose. With a little practice, it is easily possible to carry out the entire manipulations concerned with putting up and exposing a series of 6-10 preparations within an hour; while the unprotected controls are dealt with in a few minutes. As the result of an hour's work, excluding the preliminary and final subcultures, it is thus possible to make practically certain of obtaining a culture derived from a single bacterial cell, the whole process being controlled at each step under satisfactory optical conditions. We would again emphasise that it is this last requirement that appears to us to be the essential factor.

As mentioned above, the majority of our work has been carried out on paratyphoid bacilli, but we have tested the applicability of the method to certain other organisms. *B. coli*, *Staphylococcus albus*, *Staphylococcus aureus* and *Streptococcus haemolyticus* are all killed by exposure for one minute at a distance of three inches from the source of radiation, and successful isolations have been made from these organisms. Our experiments with spore-bearing bacilli are as yet too few to warrant any definite statement, but, as would be expected, the time of exposure must be very greatly increased. With regard to anaerobic organisms, we have so far attempted the isolation of single-cell cultures of *B. welchii* only. This has been successfully carried out, but the best method of ensuring the requisite conditions for growth, and the applicability of the technique to other anaerobes, have not yet been established.

We are indebted to the Medical Research Council for supplying a mercury arc with which some of these experiments have been carried out.

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EXPLANATION OF PLATE I.

- Fig. 1. Bacterial cell, showing incipient division, chosen for isolation.
 Fig. 2. Appearance presented by mercury droplet when the edge is focussed.
 Fig. 3. Appearance presented by mercury droplet when the tube of the microscope is slightly raised. The reflection of the needle-point is shown in the upper part of the droplet.
 Fig. 4. Multiplication of the protected bacterial cell after incubation.

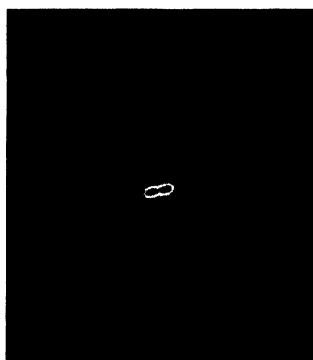


Fig. 1

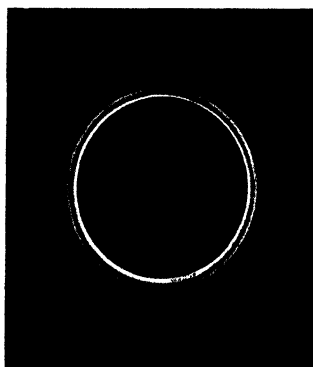


Fig. 2



Fig. 3

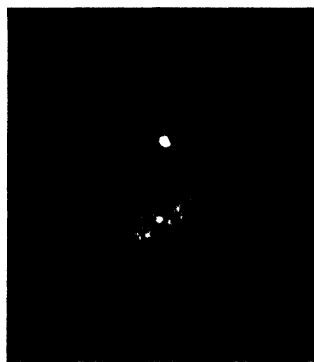


Fig. 4

THE INTERRELATIONSHIPS BETWEEN THE VARIOUS MEMBERS OF THE *B. ENTERITIDIS* —*B. PARATYPHOSUS* B GROUP OF BACTERIA.

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A REPORT TO THE MEDICAL RESEARCH COUNCIL.

(With 3 Charts.)

IN a series of investigations on the epidemic spread of bacterial infection among mice (Topley, 1919, 1921), it has repeatedly been noted that, if an epidemic be started among a mouse-population by feeding certain animals on cultures of *B. gaertner* and subsequently introducing other susceptible mice into the cage, a varying proportion of these latter may succumb to infection with an organism morphologically and culturally identical with *B. gaertner*, but differing sharply from it in regard to its agglutination reactions. This organism has been referred to in previous communications as belonging to the *B. suispestifer* group, using that term to include *B. aertrycke* and other closely related types.

The object of the investigations already reported, and of others still in progress, has been to gain some knowledge of those biological laws which must govern the spread of epidemic disease. It has therefore been necessary to attempt an answer to the question whether we should regard an epidemic, during which mice have died from infection with each of these bacterial types, as a homogeneous outbreak, or whether we must consider each infection separately.

While we are far from suggesting that the serological differences which have been demonstrated are without significance, yet we believe that for broader purposes of analysis the whole series of deaths which occur under such circumstances should be taken together, a procedure which has already been adopted in considering the results of earlier experiments (Topley, 1921).

We have been led to this view by a consideration of the available evidence with regard to the interrelationships among the complex paratyphoid group, and of the ascertained facts regarding the serological complexity of such bacterial species as the Pneumococcus and the Meningococcus. We have also, in the course of the past three years, carried out a large number of experi-

ments on points which have been brought prominently before us in our own work. It appears to us that the nomenclature and classification of the organisms of the paratyphoid-gaertner group may need some revision when viewed from a broader biological standpoint. For this reason the following facts may be worth recording.

THE EPIDEMIOLOGICAL EVIDENCE.

In general, if two epidemic diseases progress through a population during approximately the same time-interval, their course will be relatively independent, in the sense that a morbidity or a mortality curve will show individual waves each with its own maximum and minimum points. Certain individuals will contract both infections, and it is most unlikely that the progress of one infection is without influence on that of the other; but in the

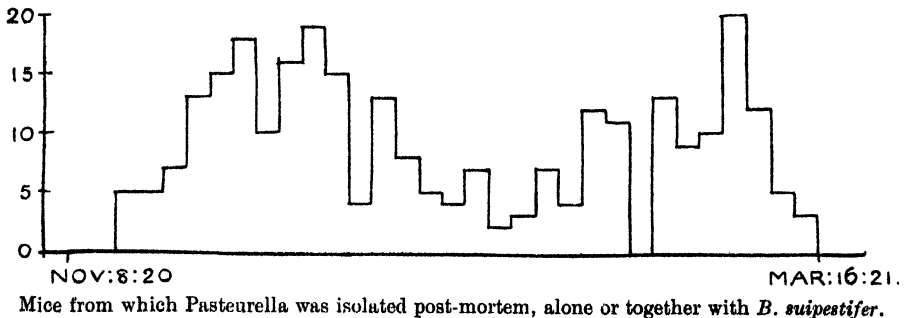
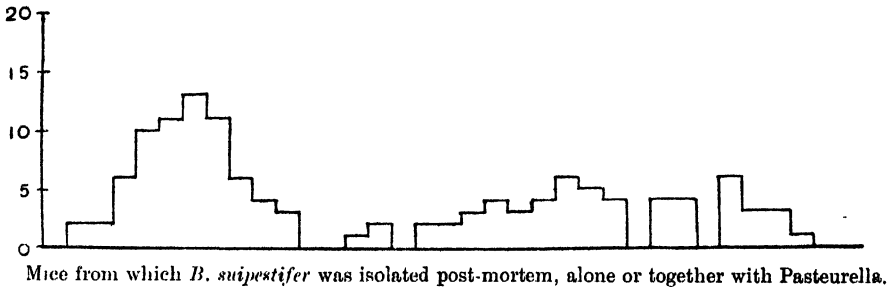
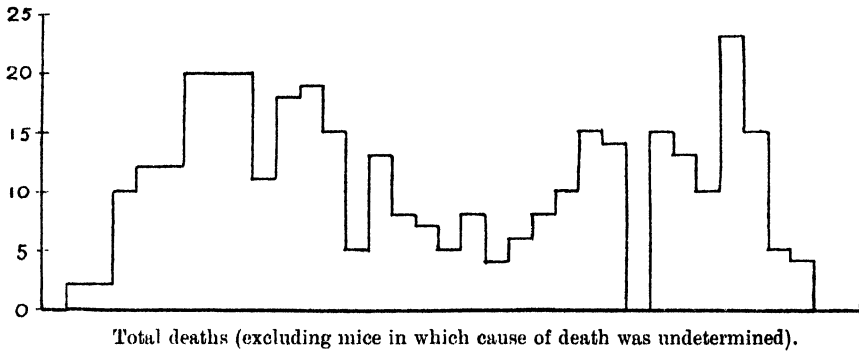


CHART I.

sense already referred to they may be regarded as separate events, and a mortality curve for the whole population concerned during the epidemic period may usually be analysed into two curves, having well-defined and separable phases.

That this is true of epidemics occurring under the experimental conditions which have obtained in our own work is shown by a consideration of Chart I, which illustrates the sequence of events following the accidental initiation of an epidemic due to an organism of the *pasteurella* group, in a cage in which an infection due to a paratyphoid organism had been progressing for many months. Throughout the period considered three normal mice were daily added to the cage. The deaths are charted in four-day intervals, and only those mice are included in which a full post-mortem examination clearly established the cause of death. Those animals in which such an examination revealed a double infection are included in the mortality chart referring to each disease.

The results show that the two waves of mortality affecting the whole population are separable into two waves of *suipestifer*-infection and two of *pasteurella*-infection. The ascent of the first *suipestifer* wave commences before that of the *pasteurella* wave; its maximum is reached earlier, and it is falling while the latter is still rising. The ascent of the second *suipestifer* wave commences before the first *pasteurella* wave has fallen to its minimum, and again its maximum point antedates that of the latter.

In Chart II similar facts are given with regard to an epidemic, which was commenced by feeding mice on cultures of *B. gaertner* and subsequently adding susceptible individuals to the cage, at first irregularly and later at a constant rate. The bacilli isolated from the tissues of these mice were identical with regard to their morphological, cultural and fermentation reactions, but agglutination tests divided them into two groups, typical *B. gaertner* strains on the one hand, and, on the other, strains which were provisionally designated as *B. suipestifer*. Three colonies from the heart and three from the spleen were examined in every case. Many mice yielded pure cultures of one or other organism, but others yielded strains of both types.

Examination of the chart shows that, in this case, the waves of mortality affecting the whole cage-population cannot be analysed into a regular series due to one type of organism, and a second series due to the other. In most of the successive waves each organism plays a part, nor is there any indication of separable maximal and minimal points. There is a general tendency for the relative frequency of isolation of the *B. suipestifer* strains to diminish as the experiment proceeds, and in the penultimate wave shown on the chart this type of organism apparently plays a very minor part. It is, however, well represented in the next and final wave.

It should perhaps be mentioned that smaller epidemics due to infection with these bacteria, and especially small outbreaks occurring spontaneously among laboratory stock, are more usually referable to organisms of one of

these serological types alone. If, however, the epidemic be of considerable proportions, and especially if large numbers of fresh susceptibles be exposed to infection, it frequently happens that both types are represented. It may be noted that, when the great majority of the mice yield strains of one type,

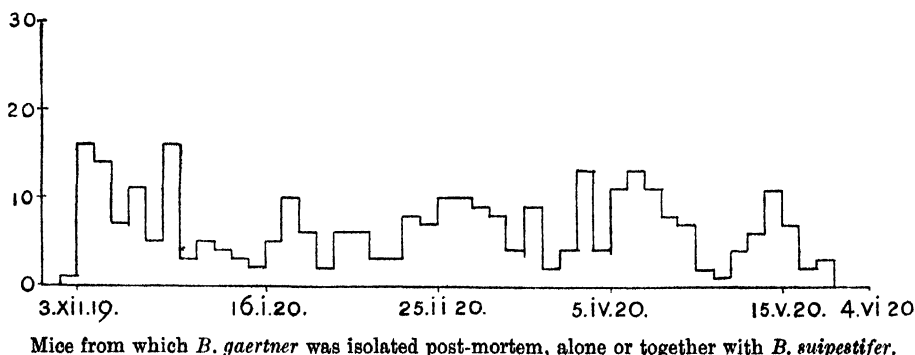
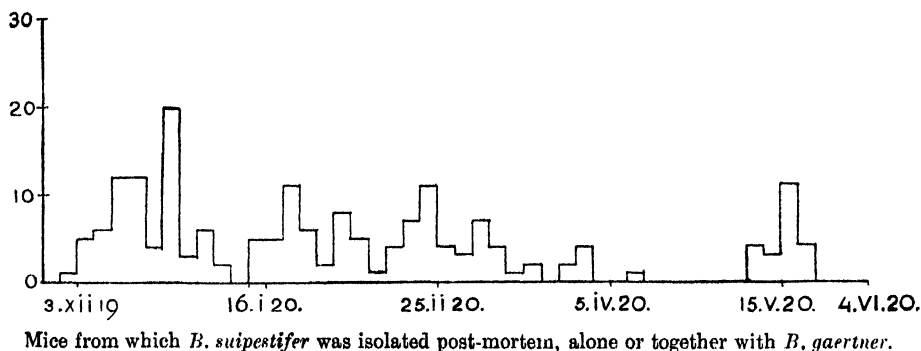
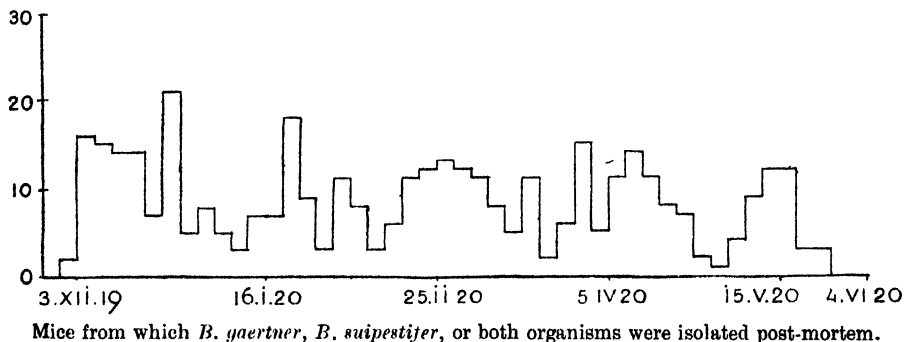


CHART II.

while strains of the other type are seldom recovered, these latter are commonly found among those animals which die in the early or closing stages of the epidemic. Chart III illustrates such distribution in two epidemics, each of which was started by feeding three mice on cultures of *B. gaertner*, and subsequently exposing other normal mice to infection.

We have not yet studied large epidemics, which have been started by infecting mice with strains of the *B. suipestifer* type; so that we do not know whether, under such circumstances, strains of the *B. gaertner* type would be frequently recovered from the tissues of those animals which succumbed. We have, however, investigated several spontaneous epidemics among mice, due to the former type of organism, but have never isolated any strain which has given the serological reactions of *B. gaertner*, although multiple agglutination with the three sera employed has been frequently observed.

It is of interest to compare these results with those observed in human epidemics of disease, and in certain outbreaks among laboratory animals, in which the causal organism is divisible into several serological types. Two bacterial parasites are especially well suited for enquiries of this kind, the Meningococcus and the Pneumococcus. The outbreaks of cerebro-spinal meningitis, which occurred among the troops in dépôts during the recent war, afforded a large amount of material for such studies. The greater part of the evidence available, from the investigations carried out in this country,

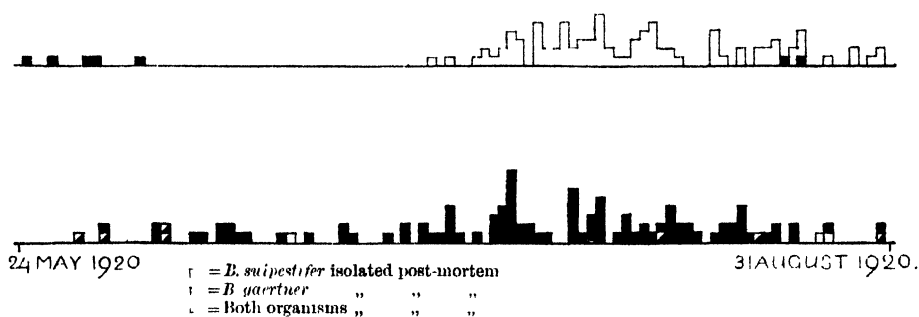


CHART III.

is contained or summarised in the series of reports issued by the Medical Research Council (1916-1920). The results recorded can be referred to only in the briefest manner, but the following facts may be regarded as definitely established. With regard to healthy carriers, it has been the universal experience of all observers that the rise in the meningococcal carrier-rate, which precedes an outbreak of meningitis, and the high level of this rate which is maintained during the epidemic period, concern not one of the serological types alone, but many or all of them; though one type usually stands out in marked predominance over the others. This multiplicity of serological types may be found not only in the same dépôt, but among men sleeping in the same hut (Glover, 1918 b).

We are thus justified in stating that the pre-epidemic stage of that process, which results in the occurrence of an outbreak of cerebro-spinal meningitis, is marked by an increase of the general meningococcal population in the naso-pharynxes of the human population concerned, and that, while one serological type of the parasite usually predominates, all types may, and

usually do, share in the invasion. When we turn to the strains isolated from the cerebro-spinal fluid of actual cases of disease we are on more difficult ground, mainly because the actual number of cases, which may be certainly grouped together as constituting a single outbreak, are in most instances relatively few. The results would seem to establish the following facts. In outbreaks involving a relatively small number of cases it is commonly found that all the Meningococci isolated belong to a single serological type. In some instances, however, cases which occurred in the same dépôt, and were closely related in point of time, were referable to different serological types¹. In such cases the predominant type of organism isolated from cases has tended also to be the predominant type found in carriers among the populations affected. This correspondence between prevalent carrier-types and prevalent case-types is well illustrated by the results obtained at Chatham by Armstrong and Tulloch², at Caterham (Glover, 1918*a*, 1920), and in the figures for the London Command in general quoted by Glover (1920).

In this connection we may note a result obtained in an experimental epidemic already reported (Topley, 1921). Fifteen mice, which had passed through a considerable epidemic in which the great majority of deaths had been due to infection with *B. suispestifer*, but in which *B. gaertner* had played a minor rôle, were killed and examined post-mortem. The animals were apparently in perfect health, and no death had occurred in the cage during the previous fourteen days. From the tissues of nine of these mice *B. suispestifer* was isolated, in eight cases in pure culture and in one together with *B. gaertner*. Thus the type of organism which was predominant during the epidemic was also more frequently isolated from the tissues of those animals which survived it.

An epidemic of cerebro-spinal fever on a far larger scale is reported by Olitsky (1919). This outbreak, which occurred among a Chinese civil population, was responsible for 1041 diagnosed cases, and Olitsky estimates that the total number affected was probably in the neighbourhood of 2500. Pure cultures of Meningococci were obtained from the cerebro-spinal fluid in 60 cases. Of these, 59 were obtained during the height of the epidemic. Agglutination tests identified 56 of these 59 strains as Parameningococci, which are stated to correspond to Gordon's Type I. The other three strains were identified as belonging to an "irregular Paratype," which is stated to correspond to Gordon's Type III. One strain was obtained from a case which occurred a few weeks after the main epidemic had come to an end. Serologically it was found to be a Meningococcus of the "normal type," stated to correspond to Gordon's Type II.

With regard to the Pneumococcus, we are faced with the same difficulties as in the case of the Meningococcus. Many of the outbreaks in which the serological type of the cocci isolated has been recorded, have involved rela-

¹ *Med. Res. Council Special Repts.* 1918 and 1920, and especially Fildes and Baker, 1918.

² *Ibid.* 1920.

tively few cases; but some records, notably those of Lister (1913, 1916, 1917) on pneumonia on the Rand, have dealt with far larger numbers of cases. It is, however, difficult to obtain information from the available records with regard to the serological type of any considerable series of strains, isolated under such circumstances that we may safely regard them as being the causative agents in a homogeneous outbreak of disease. The outbreaks of pneumococcal infection associated with the recent pandemic of influenza have afforded further evidence as to the presence of several serological types among the *Pneumococci* infecting the population concerned, but here the primary epidemic process is due to some other organism.

Some of the following facts, however, are not without significance. It is clear from the account given by Lister (1917) of the incidence of the various types of *Pneumococci* among the native mine-workers, that several types were isolated from cases occurring within a relatively short period in the same mine, which apparently formed well-defined groups. In a careful investigation among the native workers on the Crown Mines, who had received prophylactic inoculation against the three serological races, which were responsible for a majority of the cases of lobar pneumonia on the Rand, Lister (1917) investigated the type of *Pneumococcus* isolated in 80 out of 82 successive cases of lobar pneumonia. He found that the three types against which protective inoculation had been carried out were entirely unrepresented in this series of cases. Fifty of the 80 strains isolated were separable into seven distinct groups, while 30 remained unclassified.

Opie, Freeman, Blake, Small and Rivers (1919) report an outbreak of pneumonia at Camp Funston among negro drafts. Among 34 cases, in which the *Pneumococci* were investigated by agglutination, four separate groups were involved, and 13 of the 34 strains fell into the entirely heterogeneous Group IV. The many reports which have been published by American observers on the *Pneumococci* isolated during influenzal epidemics, all agree in recording several different serological types among the organisms isolated, whether from blood cultures, from sputum, or from the lung tissue at post-mortem examination. Again, while the very thorough studies of Stillman (1917) indicate unmistakably the correspondence between case-type and prevalent carrier-type of *Pneumococcus* (so far as the patient and his immediate contacts are concerned), yet they do not support the view that the case-type is always uniform, even in quite small outbreaks.

Finally, we would select for reference an interesting record of a spontaneous outbreak of pneumonia among monkeys being kept as laboratory stock. This outbreak is reported by Blake and Cecil (1920), and involved the death of 36 monkeys among 98 animals which had arrived in the laboratory in two batches. *Pneumococci* were isolated in 28 cases, and all were found to belong to the heterogeneous Group IV. Agglutinating sera were prepared against three strains, and with these sera 21 of the 28 strains were retested; nine fell into one group, five into another and seven remained unclassified.

The conclusions which appear to be justified from a consideration of the facts recorded in this section may be summarised as follows. The available evidence regarding epidemics of disease, caused by a bacterial species which shows subgroups separable from one another by serological reactions, indicates that representatives of all or many of these subgroups take part in the essential process on which the epidemic depends. In small outbreaks it is a common experience for all clinical cases of the disease in question to be referable to infection by organisms belonging to the same serological subgroup, but in larger outbreaks this uniformity is frequently absent. If parallel examinations be carried out on healthy contacts and non-contacts, living in the epidemic area, it will almost always be found that the serological subgroup responsible for all, or for the majority of the clinical cases, is the predominant group among the total number of strains of the parasitic species concerned, which are isolated from the population at risk.

The phenomena observed in experimental epidemics, in which *B. gaertner* and *B. suipestifer* have played a causative rôle, would suggest that these organisms are related to one another in the same way as are the serological subgroups of such bacterial species as the *Pneumococcus* or the *Meningococcus*.

EVIDENCE FROM THE IDENTITY OF THE LESIONS PRODUCED.

In the course of the past three years we have carried out post-mortem examinations on over 2000 mice which have died as the result of infection with *B. gaertner* and *B. suipestifer*. We do not propose to give any detailed account of our study of the lesions observed beyond stating that the findings of previous workers have been largely confirmed, and in some directions amplified. We may emphasise however that certain of the tissue changes, and especially perhaps the multiple necrotic areas so frequently found in the liver, are striking and typical. Examination of this considerable number of mice has entirely failed to reveal any constant difference, which would separate those animals which have died from infections with organisms of one serological type from those which have succumbed to the attack of the other.

EVIDENCE WITH REGARD TO THE SEROLOGICAL RELATIONSHIP OF *B. GAERTNER* AND BACILLI OF THE *SUIPESTIFER* GROUP.

There has been a general consensus of opinion among most observers that, while *B. paratyphosus* B, *B. aertrycke* and *B. suipestifer* show close serological relationship, so that absorption tests are frequently necessary to separate them on the grounds of their agglutination reactions, *B. gaertner* is sharply marked off from the members of this group by its direct response to agglutination with specific sera. Evidence has not, however, been wanting that this separation may not be so absolute as has been supposed. Sobernheim and Seligmann (1910) studied certain strains of *B. paratyphosus* B, which became inagglutinable with their specific antiserum, and at the same time

acquired the property of being agglutinated with a gaertner antiserum. The serum produced by inoculation of these strains agglutinated *B. paratyphosus* B but not *B. gaertner*.

Since the time when we first noted that organisms with the serological reactions of *B. suipestifer* were being isolated from mice dying during an epidemic started by feeding with *B. gaertner*, it has been our routine procedure to test each strain isolated against high-titre agglutinating sera prepared against *B. gaertner*, *B. aertrycke* (Mutton) and one of the earliest strains of the *B. suipestifer* type isolated in the course of this work. Blood from the heart and spleen-pulp have been smeared directly on to plates of agar or MacConkey's medium, or primary broth cultures from these sources have been plated out after preliminary incubation. From the plate-cultures so obtained from each mouse, three colonies derived from the heart and three from the spleen have been subcultured into broth. After 24 hours' incubation at 37° C. these subcultures have been killed by diluting with normal saline containing 0.5 per cent. formalin and heating to 55° C. for one hour, a second subculture having first been made in order that the strain might be available for further study if desired. Heat has been employed in addition to formalin for killing the cultures because, when several hundred strains were being examined weekly, it was impossible to test each suspension for sterility by subculture. Previous experience had shown us that the procedure adopted ensured uniform sterility without rendering the suspensions in any way unsuitable for our purpose.

From the first, it was obvious that the serological complexity of the strains isolated was considerable. Almost every imaginable variety of result has been obtained in direct agglutination tests, and there is little doubt that, had additional sera been employed, still greater complexity would have been revealed. A large number of strains, on first isolation, agglutinate to titre with the gaertner or suipestifer serum and are quite unaffected by the other two. Many strains agglutinate with two of the three sera, and when this is the case the combination is almost always gaertner and aertrycke, or suipestifer and aertrycke. Very few suspensions have agglutinated with gaertner and suipestifer sera, while remaining unaffected by the aertrycke serum; and further subcultures from such strains have always resulted in the isolation of both the gaertner and suipestifer types, so that they were presumably mixed strains from the outset. Not infrequently a strain has been agglutinated by all three sera. When multiple agglutination occurs, the strain concerned may be agglutinated to titre by all three sera, much more commonly by two of them; or only one serum may produce this result, the others giving agglutination in lower dilution. All strains which have behaved in this way have been further subcultured and retested. The results have been uniform and striking. In every case, a strain which on first isolation agglutinated with more than one of the three sera employed has, after a very varying number of subcultures, yielded a strain which has agglutinated to titre with one serum

and remained entirely unaffected by the others. It may be noted here that we have never isolated a strain which has permanently displayed the serological characters of *B. aertrycke* (Mutton). Still further subcultures may again yield strains showing multiple agglutination, but if the process be persisted in the cultures will again revert to the specifically agglutinable type. Moreover, strains of *B. gaertner* or *B. suispestifer*, which show typical agglutination to titre with one of the corresponding antisera, never give rise to strains showing typical agglutination to titre with the other. In practice, it is usually possible to distinguish the serological type to which a strain actually belongs at the first agglutination test. Either it will agglutinate to titre with the gaertner or suispestifer serum, but not with the other two sera, or it will form large flocculi with one serum, and much smaller and tighter clumps with the other. In such cases further subculture always yields a strain agglutinating only with that serum, which originally caused agglutination to titre or the formation of large flocculi. This variation in the type of the clumps produced has been noted by many workers, and has recently been extensively studied by Arkwright (1921) in his investigations on saline-agglutinable strains.

The figures quoted in Table I may be considered typical of the results we have obtained. The upper horizontal line gives the agglutination titres, obtained with one of three colonies picked from a plate inoculated with a 24 hours' broth culture from a mouse, which died during an epidemic of mouse-paratyphoid. Below are given the results obtained on retesting subcultures

Table I.

Date	Strain	Sera		
		Gaertner	Aertrycke (Mutton)	Suispestifer
9. xii. 19	Sp. A	6400	6400	6400
6. ii. 20	Sp. A1	6400	800	800
	Sp. A2	6400	1600	1600
	Sp. A3	6400	800	—
	Sp. A4	6400	—	400
	Sp. A5	6400	1600	400
13. ii. 20	Sp. A1 A	6400	3200	1600
	Sp. A1 B	6400	—	—
	Sp. A1 C	6400	1600	800
	Sp. A1 D	6400	—	—
	Sp. A1 E	6400	—	—
17. ii. 20	Sp. A1 A1	6400	—	—
	Sp. A1 A2	6400	—	—
	Sp. A1 A3	6400	—	—
	Sp. A1 A4	6400	—	—
	Sp. A1 A5	6400	—	—

Note.—The five strains tested on 6. ii. 20 were obtained from five separate colonies from a plate inoculated with strain Sp. A tested on 9. xii. 19. Similarly the five strains tested on 13. ii. 20 were derived from strain Sp. A1 tested on 6. ii. 20, and those tested on 17. ii. 20 from Sp. A1 A6 tested on 13. ii. 20. The figures indicate the highest dilution of serum giving well-marked agglutination.

— = No agglutination at a dilution of 1/400.

of this strain at later dates. The strain was subcultured once only between successive tests, and plate cultures were prepared from which colonies were picked after 24 hours.

Further investigation soon made it clear that the occurrence of strains showing multiple agglutination was not dependent on passage through the tissues of the mice. A culture of *B. gaertner* obtained from the Lister Institute in 1918, which on previous examination had agglutinated with its specific antiserum but not with the other sera employed, was frequently subcultured in broth over a period of many months.

Plate cultures were prepared at intervals from which ten separate colonies were picked, subcultured into broth, and tested against the three antisera indicated above. It is unnecessary to tabulate the results, for they were in all essential respects similar to those already recorded. Multiple agglutination was frequently met with, sometimes to such an extent that there was no indication of the real nature of a given bacterial culture from the results of a single test. In this series of experiments it was by no means unusual to find strains, derived from this culture of *B. gaertner*, which entirely failed to respond to the specific antiserum, but which agglutinated to a high titre with the aertrycke serum employed. At other times, strains were obtained which failed to agglutinate with any of the three sera, but further subculture invariably revealed the true nature of the strain concerned.

A single instance of this kind may be quoted. From a subculture of the original strain, a culture was prepared from a single bacterial cell by a method described elsewhere (Topley, Barnard and Wilson, 1921). From this culture a plate was inoculated from which ten colonies were picked. The resulting broth cultures were tested against the three antisera, but gave no trace of agglutination with any of them. Two of these were further subcultured and treated in the same manner. In one case all the ten subcultures agglutinated with the gaertner and the aertrycke serum, but while they agglutinated to titre with the latter they were only acted upon by their specific antiserum in much lower dilutions. In the second case three out of six subcultures were agglutinated to a high titre by the aertrycke serum, but not at all by the specific antiserum. One of these latter strains was again plated out, and six colonies were subcultured and retested. They all agglutinated to titre with the gaertner serum, but were only acted upon by the aertrycke serum in lower dilutions.

The real nature of such a gaertner strain, which has become inagglutinable by its own antiserum while responding to the action of an aertrycke serum, is at once revealed by absorption tests. Absorption of an aertrycke serum by such a strain leaves the titre against *B. aertrycke* entirely unchanged, while diminishing or abolishing its action on the abnormal gaertner strain. If, however, the same strain be employed to absorb a gaertner serum, the agglutinins both for *B. gaertner* and for the aberrant strain may be entirely removed.

While it would be wearisome to repeat details of similar variations among strains which originally showed multiple agglutination, but finally reacted as pure *suipestifer* strains, or starting as pure *suipestifer* strains came to show multiple agglutination, it may be stated that all the observations noted above have been paralleled among such strains, with the exception that we have not carried out absorption experiments in these cases.

In view of the recent observations of Arkwright (1921) on the separation of saline agglutinable and inagglutinable strains of *B. dysenteriae* (Shiga), it was natural to enquire whether the variant strains we have studied showed similar phenomena. Our technique was not well-suited for revealing such relations, had they been present, but it was possible to make certain observations along these lines. Thus the roughness or smoothness of the colonies on plates, and the type of growth in the broth subcultures, were noted in large series of cases. The form of the colonies varied widely, and in general a given plate culture tended to show all rough or all smooth colonies, but either character might be continued through many generations, or suddenly give place to the other. So many intermediate types of colonies were met with that a classification into rough and smooth was frequently very arbitrary. The growth in broth was in most cases of the type usual with organisms of this group, with uniform turbidity and slight deposit. Occasionally, however, an entirely different kind of growth occurred, a heavy, almost granular deposit collecting at the bottom of the tube, while the supernatant fluid remained clear or only slightly cloudy, but often showed a film of surface growth. On shaking such a culture a uniform suspension was obtained, which proved quite suitable for agglutination tests. This type of growth was particularly common after prolonged subculture, with frequent transplants from broth to broth. It was especially with such strains that the more aberrant type of agglutination was likely to occur, and it seemed at first as though some relation might be established between the two phenomena. More extended experience has shown that, while such cultures are more likely to depart widely from the normal type in regard to their agglutination reactions, especially in the direction of loss of specific agglutinability associated with the acquirement of agglutinability by a heterologous serum, yet they often react in a perfectly normal manner; while equally wide variations may occur in cultures which show a perfectly normal type of growth. We have entirely failed to establish any relation between the roughness or smoothness of the colonies and the subsequent behaviour of subcultures in agglutination tests.

To sum up these results, it may be said that examination of large numbers of strains has shown that the serological distinction between *B. gaertner*, *B. aertrycke* and *B. suipestifer* is less sharp than has been commonly supposed, so far at least as direct agglutination tests are concerned. An organism of one type may acquire the property of being agglutinated to titre by one or both of the heterologous sera. It may become inagglutinable by its specific antiserum at the same time as it acquires the property of agglutinability by

a serum specific against one of the other types, though this is a far less common occurrence. While this interrelationship is clearly shown by direct agglutination, the same procedure, repeated on many successive subcultures, always reveals the true nature of the strain, and the indication so obtained is confirmed by absorption tests.

THE QUESTION OF MUTABILITY.

The observations described above raise once again the question whether the serological types dealt with are bacterial mutants, in the sense that one type may be repeatedly derived from another under natural or experimental conditions. It would be an immense satisfaction to obtain an unequivocal answer on this point, but no adequate evidence has yet been brought forward. The matter has been debated, as regards this group of organisms, by Schmitt (1911), Mühlens, Dahm and Fürst (1909), Gurney-Dixon (1919), Jordan (1920), and others.

We have ourselves observed large numbers of strains over considerable periods of time, up to two years; and have subjected many of them to highly abnormal conditions of environment, such as prolonged storage in distilled water or normal saline, growth at 45° C. over many months, and prolonged growth on agar at different temperatures, with subcultures of the papillae from the large colonies which form under these conditions. Without entering into details we may say that we have completely failed to obtain a strain which, after adequate examination, could be placed in any other group than that proper to the strain from which it was originally derived.

We have also carried out feeding experiments on considerable numbers of mice, testing the strains recovered from the internal organs after death, or obtained by killing the animals while in apparently good health at various intervals after feeding. In the majority of cases, mice fed on cultures of *B. gaertner* have yielded cultures of this type alone, and similarly with mice fed on cultures of *B. suispestifer*. Occasionally, however, we have isolated one of these serological types from the tissues of a mouse fed on cultures of the other. It is clear that such instances of the isolation from animal tissues of a strain, showing different serological reactions from those of the strain administered, cannot be regarded as satisfactory evidence of the mutational origin of the former strains.

THE RELATIONSHIP BETWEEN THE MEMBERS OF THE PARATYPHOID-ENTERITIDIS GROUP.

This thorny question has long been a subject of controversy between different schools of bacteriologists. Any adequate résumé of the literature would be far beyond the compass of this communication. The excellent survey of Uhlenhuth and Hübener (1913) gives a summary of the evidence acquired prior to that date, and considers it from the point of view of those who would divide the group into two subgroups only, one confined to

B. enteritidis (Gaertner), and the other including *B. paratyphosus* B, *B. aertrycke*, *B. typhi-murium*, *B. suispestifer*, and a host of allied organisms recovered from the tissues and excreta of sick or healthy animals.

Bainbridge (1912) and Bainbridge and O'Brien (1911) have insisted on the distinction which may be drawn between *B. paratyphosus* B, on the one hand, and *B. aertrycke* and *B. suispestifer* on the other, by means of absorption tests. The identity which they found between the two latter types is probably invalidated, as pointed out by Tenbroeck (1920 *a*), by the fact that their *B. suispestifer* strains were of German origin, and may well have been of the type which would be referred to by recent American observers as swine-typhus bacilli. The results of Bainbridge and O'Brien have, however, led to a very general use of *B. suispestifer* and *B. aertrycke* as synonymous terms in this country; and it is in this wide sense that the former has hitherto been used by us. Schütze (1920), by an extended use of absorption tests, has recently erected several additional subgroups within the general *B. paratyphosus* B group.

The most valuable recent additions to our knowledge of this complex group have been derived from the studies of various American workers, including among others, Jordan (1917, 1918 *a*, 1918 *b*, 1920), Jordan and Victorson (1917), Krumwiede and Kohn (1917), Krumwiede, Pratt and Kohn (1916 *a*, 1916 *b*, 1917), Krumwiede, Kohn and Valentine (1918), Krumwiede, Valentine and Kohn (1919), Smith and Tenbroeck (1915), Tenbroeck (1918 *a*, 1918 *b*, 1920 *a*, 1920 *b*), Murray (1919), Muslow (1919), Rettger and Koser (1917), Meyer and Boerner (1913), Good and Corbett (1913), Gage and Martin (1916), and Winslow, Kligler and Rothberg (1919). In the following brief summary only those papers are referred to, which deal with those organisms that have been less thoroughly studied, or with facts recently brought forward.

The careful studies of fermentation reactions and serological relationships, which have been undertaken, have left a picture of the whole group which may be presented somewhat as follows. The organisms which comprise it, with certain exceptions noted below, have the following characters in common. They are gram-negative, non-sporing, motile bacilli. They ferment dextrose, maltose, mannite, xylose and rhamnose with the formation of acid and gas. They do not ferment lactose, saccharose, salicin, raffinose, dextrin nor inulin. They produce transient acidity in litmus-milk, but later give rise to a markedly alkaline reaction. They reduce neutral red. They do not form indol nor do they liquefy gelatine.

This group, according to the researches so far conducted, may be split up into the following subgroups:

(*a*) *B. paratyphosus* B possesses the general characteristics of the group, rapidly ferments dulcitol, ferments arabinose and inositol, produces blackening of lead acetate and reduces the fuchsin in the serum-water medium recommended by Krumwiede, Pratt and Kohn (1917). It is readily distinguishable

from *B. enteritidis* (Gaertner) by direct agglutination, and from other members of the group either by direct agglutination or by absorption.

(b) *B. suipestifer* possesses the general characteristics of the group, ferments dulcitate slowly or not at all, does not ferment arabinose nor inosite, does not blacken lead acetate medium, and does not reduce the fuchsin in serum-water medium. This organism may be distinguished from *B. enteritidis* by direct agglutination, and from *B. paratyphosus* B and allied species, sometimes by direct agglutination but more commonly by absorption tests. According to Reed and Carroll (1900) and Tenbroeck (1920 b) *B. icteroides* (Sanarelli) belongs to this subgroup.

(c) *B. paratyphosus* C, so named by Hirschfeld (1919), who isolated it during an epidemic of paratyphoid fever in the Balkans, but more generally known as Hirschfeld's Bacillus, to distinguish it from Uhlenhuth's *B. paratyphosus* C, to which apparently it has no relation. It has all the general characteristics of the group, ferments arabinose and dulcitate and blackens lead acetate. As regards its fermentation reactions therefore it cannot be differentiated from *B. paratyphosus* B and *B. enteritidis*. A recent study by Tenbroeck (1920 b) has shown, however, that serologically it is identical with *B. suipestifer*, as judged both by direct agglutination and by absorption tests.

(d) *Paratyphoid bacilli of animal origin*. This large subgroup includes a great number of strains derived from the tissues and excreta of animals, either healthy or diseased. There is good evidence that the bacteria concerned are in many cases the actual cause of the disease in question, while in other cases they should perhaps be regarded as secondary invaders. It is also established that several animal species are subject to severe epidemics, in which these organisms play a causal rôle. These strains possess the general characters of the group, they ferment dulcitate and arabinose, while their reaction in inosite appears to be irregular. It is noteworthy that Tenbroeck (1920 a) obtained indefinite results with this substance, and was unable to establish any differentiation between *B. paratyphosus* B, *B. enteritidis* (Gaertner) and these animal paratyphoid bacilli on the basis of their fermentation reactions. They blacken lead acetate medium and reduce the fuchsin in serum-water medium. They are distinguishable from *B. gaertner* by direct agglutination, and from other members of the group by absorption tests. When specific sera are prepared against individual strains of this group, there appears to be a correlation between the degree of agglutination and host-origin. It is probable that *B. aertrycke* belongs to this subgroup, and also those *B. suipestifer* strains of German origin which were studied by Bainbridge and O'Brien (1911). The bacilli isolated from mice dying during our own experiments, and hitherto referred to as *B. suipestifer*, have all the characteristics of this subgroup and should be placed in it.

(e) *B. enteritidis* (Gaertner) possesses all the characteristics of the group. It ferments dulcitate and arabinose, but not inosite; though the value of this latter substance for the purpose of differentiation has still to be firmly estab-

lished. It blackens lead acetate medium and reduces the fuchsin in the dextrose-serum-water medium of Krumwiede, Pratt and Kohn. It is separable from most other members of the group by direct agglutination, but shows definite serological relationship with *B. pullorum* and *B. gallinarum*, from both of which it may be separated by absorption tests (Smith and Tenbroeck, 1915, and Muslow, 1919).

(f) *B. abortus equi*, a paratyphoid organism which appears to be responsible for epidemic abortion in mares, has been studied by Smith (1893), Kilbourne (1893), Good and Corbett (1913), de Jong (1913), Meyer and Boerner (1913), van Heelsbergen (1914), Murray (1919), and Fitch and Billings (1920). It possesses the general characters of the group, ferments dulcitol and arabinose but not inositol, fails to reduce lead acetate and does not reduce the fuchsin in serum-water medium. On agar it forms a distinctive, dry and brittle growth. Serologically, it is readily distinguishable from other members of the group.

(g) *B. pullorum* is regarded by many authorities, but not by all, as the cause of bacillary white diarrhoea in chicks. It was isolated by Rettger (1900), and has been studied by Smith and Tenbroeck (1915), Gage and Martin (1916), Rettger and Koser (1917), Krumwiede and Kohn (1917), Hadley, Caldwell, Elkins and Lambert (1917), Hadley, Elkins and Caldwell (1918), Muslow (1919) and others. According to the reports of most of those who have studied this organism, it differs from the groups mentioned above by its non-motility, its initial production of acidity in milk followed by a very slow change to alkalinity, and by its failure to ferment xylose and maltose. Muslow (1919), however, reports uniform production of acid in xylose, with very variable gas-production, and doubts the reliability of the reaction in litmus-milk, as a means of differentiating between this organism and *B. gallinarum*. It ferments arabinose, but not dulcitol nor inositol. As regards its action on lead acetate there is a distinct conflict of statement. Muslow (1919) reports that all strains examined by him produced rapid blackening. Winslow, Kligler and Rothberg (1919), summarising and confirming the results of other workers, report negative reactions. These authors, on the grounds of the reaction in litmus-milk and the absence of fermentation of xylose, would place this organism in the same group as *B. paratyphosus* A, rather than in the *B. paratyphosus* B-*B. enteritidis* group; and there is clearly much to be said for this classification. Serologically, however, *B. pullorum* appears to be identical with *B. gallinarum*, as tested by direct agglutination or by absorption of agglutinins. Both these organisms show a close serological relationship to *B. enteritidis* and also to *B. typhosus*, absorption being required to differentiate them from these organisms. There is also a definite serological relationship between *B. pullorum* and *B. gallinarum* on the one hand, and *B. abortus equi* on the other, but direct agglutination generally affords a sufficient means of differentiation.

(h) *B. gallinarum*, described by Klein (1889) as the cause of an epidemic

of fowl-typhoid in England, is almost certainly identical with the bacillus described by Moore (1895) as *B. sanguinarium*, and has since been studied by several workers, including Pfeiler and Rehse (1913), Smith and Tenbroeck (1915), Pfeiler and Roepke (1917), Hadley, Caldwell, Elkins and Lambert (1917), Hadley, Elkins and Caldwell (1918), and Muslow (1919). It departs from the general characters of the group by being non-motile, by failing to produce gas in the various test-media and by attacking dextrin. On the other hand, it produces the typical alkaline reaction in litmus-milk and ferments xylose. For these reasons Winslow, Kligler and Rothberg (1919) are inclined to place it in the group with *B. paratyphosus* B and *B. enteritidis*, in spite of the absence of gas-formation. It ferments arabinose and dulcitol but not inositol. With regard to the blackening of lead acetate medium, there is the same conflict of opinion as in the case of *B. pullorum*. Its serological reactions have already been discussed, and it is somewhat surprising that organisms, showing such wide differences with regard to their fermentation reactions as do *B. pullorum* and *B. gallinarum*, should show serological identity.

DISCUSSION.

It has been suggested above that the two types of organism isolated during the course of experimental epidemics in mice, one of which has given the serological reactions of *B. enteritidis* (Gaertner) and the other those of the animal-paratyphoid group, might be regarded on epidemiological grounds as being related to one another in the same way as are the various serological types of Meningococci or Pneumococci. It has been further shown that the demarcation between the two types, afforded by agglutination tests, is not so sharp as has been supposed; though we have obtained no evidence suggesting that either type permanently loses its serological characters, or exchanges them for those of the other.

A large number of strains belonging to each type have been tested with regard to their fermentation reactions. All test-substances recorded by recent observers have been employed, with the exception of inositol, and of the dextrose-serum-water with the Andrade indicator. We were unable to obtain a supply of inositol at the time when the fermentation reactions were being carried out, and the results recorded by Tenbroeck (1920 *a*) seemed to us to throw some doubt on its reliability as a means of differentiation. In all the media we employed the reactions of all strains have been identical, and have conformed to those generally ascribed to the subgroups in question.

Under these circumstances it would appear that the relationship between these two bacterial types would be most accurately reflected in the nomenclature by giving them a common name, and indicating the serological subgroup by a convenient suffix. The existence of this relationship has, indeed, been fully recognised in a recent report dealing with bacterial classification (Winslow, Kligler and Rothberg, 1919), in which *B. enteritidis*, *B. suispestifer* (strictly so-called), the *B. paratyphosus* B forms and *B. gallinarum* are in-

cluded in a separate subgroup of the colon-typhoid group of bacteria. It appears to us that the actual relationship is closer than is suggested by such a grouping. Employing the terminology which is, almost inevitably, adopted in bacteriological classification, we would suggest that a single "specific" name be given to the organisms comprising certain of these subgroups. From the strictly scientific point of view, the use of the term "species" and the application of specific names are, perhaps, hardly to be defended, when we consider our almost complete lack of knowledge of the actual life-history of the organisms with which we are dealing. Though the terms we use suggest analogies between the groupings we recognise and those set up in other branches of biology, yet the characters upon which our classification is based may be fundamentally different.

With these reservations, we may call to mind certain resemblances between the relationships existing among bacterial types, and those known to obtain elsewhere in nature. The larger groups of the Linnaean species are now generally acknowledged to be divisible into very numerous elementary species, as was first urged by Jordan (1853), and later insisted upon as a fundamental phenomenon by de Vries (1901). Yet the reality of the Linnaean species has not been seriously questioned, nor would a classification which raised elementary species to specific rank, as ordinarily understood, yield a possible nomenclature for systematic use. It is at least tempting to draw an analogy between serological types of bacteria and elementary species, as understood by de Vries. No one who has had extended experience with such bacterial groups as the Meningococci, the Pneumococci and the paratyphoid bacilli, will readily deny the reality of serological differentiation. The question is rather the significance which is to be attached to the differences so demonstrated. It appears to us that there is evidence in support of the view that the unit-group of such bacteria, which functions in natural parasitism, is larger than the serological subgroups, and probably includes many of them.

It seems possible that the most urgent requisite for a better understanding of bacterial parasitism is an increased knowledge of bacterial ecology. For this reason it would perhaps be better to note those minor yet constant differences, which undoubtedly occur in strains which are perpetuated under laboratory conditions, without necessarily utilising them in systematic classification. The present tendency appears definitely to be in the direction of testing bacterial strains as to their action upon an ever-widening range of possible food materials, and subjecting them to more and more subtle serological tests, giving to each strain which is separable by such means the rank of type, race or species as the case may be, and in most cases bestowing upon it a distinctive name.

We are far from minimising the value of such detailed studies. We have, indeed, obtained experimental evidence, which would seem in some measure to explain the significance of such serological subgroups in the spread of bacterial infection. This evidence we hope to publish in a future report. The

question immediately before us, however, is one of classification, and especially of nomenclature. The matter is to some extent urgent, for the present condition is chaotic; and the recent reports of the American Society of Bacteriologists (Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith, 1917) indicate clearly that some attempt at systematisation, however provisional and imperfect, will have to be made. It is from this point of view that we would plead for a broad outlook, so that our nomenclature may indicate those groupings which are most fundamental in the natural existence of the organisms which we study, and may not depend mainly on small differences observed under artificial cultivation. It would perhaps be an advantage if the Linnæan admonition "*Varietates levissimas non curat botanicus*" were borne in mind by the bacteriologist, so far at least as classification is concerned.

We are inclined to uphold the view that the large group of bacteria, which we have been considering, including at least *B. paratyphosus* B, *B. enteritidis* (Gaertner), Hirschfeld's bacillus, *B. aertrycke*, and the majority of the paratyphoid organisms isolated from animals, should receive a single specific name. If we include *B. enteritidis* in this group, and it is our main thesis that it should be so included, then this name holds priority over all others, with the exception of *B. suipestifer*. It appears to us that the latter title is far less suitable, for at least two reasons. It was applied to the organism in question by Salmon and Smith (1886) in the belief that it was the specific cause of swine-fever, so that its retention would perpetuate a view now generally held to be incorrect. Again, while we should be inclined to make the specific group as wide as possible, it must be recognised that the constancy of those fermentation reactions, which differentiate the true *B. suipestifer* from *B. paratyphosus* B and *B. enteritidis*, may possibly be held to necessitate the inclusion of the former in a different specific group. We would therefore suggest that the name of *B. enteritidis* be employed as a specific title for the bacterial types referred to above, with the possible exclusion of *B. suipestifer*, and the probable exclusion of *B. pullorum* and *B. gallinarum*.

It would be desirable to add to the specific name another, indicating the serological subgroup to which a strain belongs. This subgroup might be regarded as a variety. Thus we might employ the names Gaertner, Schottmüller and Hirschfeld to denote the three well-defined subgroups discovered by these observers, while the names *suipestifer* and *aertrycke* might be used to denote these two varieties, though the latter term would then have to include a large number of paratyphoid strains of animal origin. It would be a distinct advantage if we could make the names of our varieties denote their host-origin, and with further knowledge this may become possible, but at present some of the best marked serological differences seem to cut clearly across the lines of specific parasitism.

CONCLUSIONS.

For the reasons set out above, it seems probable that the relation between *B. enteritidis* (Gaertner) and many of the members of the *B. paratyphosus* B group of bacteria is similar to that existing between the serological subgroups of such bacterial species as the Meningococcus or the Pneumococcus. It is therefore suggested that, for purposes of classification and nomenclature, *B. enteritidis* should be included in this group, and that its name might well be applied to the whole group of which it forms a part, both on account of priority and of suitability. The subgroup, or variety, to which a given strain belongs, provided that this is sufficiently definite and constant, should be indicated by adding a name designating the subgroup or variety in question.

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A PRELIMINARY STUDY OF THE EPIDEMIOLOGY OF RHEUMATIC FEVER.

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THERE seems to be considerable evidence in favour of the view that the immediate cause of this acute infective fever is an organism in the form of a streptococcus although there does not yet appear to be unanimity as to the specific characteristics of the latter. With regard to the secondary or indirect or predisposing causes of the complaint, the agencies which influence favourably the development and activity of the organism and consequently the prevalence of the disease, there has been a definite tendency in recent years not only to abandon the hitherto prevailing opinion on the influence of certain meteorological factors, namely, that increase in the amount of rainfall and decrease in the temperature are generally associated with increased incidence of the disease but even to assert that the action of these factors is the reverse of this.

Amongst those who have contributed to the study of the influence of meteorological conditions on the prevalence of rheumatic fever is Sir Arthur Newsholme (1895), whose Milroy Lectures were devoted in part to this section of the subject. This author provides evidence which appears to be at variance with the view that excessive prevalence of rheumatic fever is associated with wet weather and dampness of soil and avers that every statistical fact regarding rheumatic fever contradicts this. He concludes that, as a rule, a heavy annual rainfall is associated with a low amount of rheumatic fever and a small rainfall with an excessive amount though there is no exact proportion observable between the two and further that two or three years of excessive or deficient rainfall are more potent than a single year. He expresses the opinion that deficient rainfall exerts its action from its influence on the level of the ground water and that, while the development of the causal saprophytic organism is favoured by a dry subsoil and a low level of ground water it appears to be drowned out in wet years. As regards the relationship of rheumatic fever to air temperature he states that on the whole it would appear that a high temperature is in England favourable to an increase of rheumatic fever though there is no definite proportion between the two; also that great epidemics of rheumatic fever occur only when the mean temperature of the soil is exceptionally high, *i.e.* over 50° F., and that the greatest epidemics occur when the mean temperature has been thus high for two or three years in succession. Dr Gabbett (1883) from a consideration of the statistics of

admissions to the London Hospital for the period 1873-81 concludes that it is difficult to trace any connection between the state of the weather and the prevalence of the disease in individual years, but that cases of the disease are very numerous at that period of the year during which there is usually a co-existence of low temperature and heavy rainfall, viz. the end of autumn. Drs Greenwood and Thompson (1907) have also investigated the relationship of meteorological factors to the statistics of admissions for rheumatic fever to the London Hospital but for a longer period than Gabbett, namely 1873-1903 inclusive. They state that while the results are not conclusive, a circumstance which they attribute to some extent to the selective character of hospital admissions in general, the inference to be drawn from the correlation between the incidence of disease and rainfall for August—the only month for which a significant coefficient value is found by them—is that rheumatism is associated with dry weather. No definitely significant correlation is found to exist for any month between the admissions to the hospital for rheumatic fever and the mean monthly temperature. Dr Longstaff (1901) concludes that the geographical distribution of rheumatic fever gives no support to the old idea that it is mainly caused by cold, wet, or chills, but that rheumatic fever shows a rough inverse relationship to rainfall and that a dry summer appears to favour an epidemic of rheumatic fever though it is not invariably followed by one. Sir William Church (1906) states that the statistics of the British army show that it is impossible to associate the number of attacks of rheumatic fever with any peculiarity of climate, but that chill is probably a most important factor in determining an attack of rheumatic fever is supported to a certain extent by the geographical distribution of the disease. There is, however, he thinks, some evidence that high and dry lands, where the temperature varies between wide limits in the 24 hours, appear to be particularly conducive to rheumatic fever. F. J. Poynton (1908) draws attention to the frequency of association of rheumatism with damp houses in a series of cases investigated by him. He suggests that the hereditary factor is important but expresses some uncertainty as to what circumstances assist the pernicious effect of the microorganism. Whipman (1888) in his analysis of 655 cases of acute rheumatism in the report of the Collective Investigation Committee of the British Medical Association does not find much definite or reliable information regarding the exact meteorological conditions which influence the prevalence of the disease, although most cases appear to begin in damp and wet or cold and cloudy weather.

The present paper is based on the results of an investigation into the geographical distribution and the secondary or predisposing causes of rheumatic fever in Great Britain. The data that have been utilised are the numbers of deaths of persons from rheumatic fever and rheumatism of the heart in the different age-groups in the registration counties of England and Wales for the period 1901-10 as recorded in the decennial supplement to the report of the Registrar-General of England and the figures for the deaths

amongst persons from rheumatism (including rheumatic fever) in certain groups of registration counties in Scotland for the period 1881-1900, which have been extracted and compiled from the supplements (38th and 48th) to the reports of the Registrar-General for Scotland. These data may be considered to be defective inasmuch that the figures used relate to the numbers of deaths from rheumatic fever and not to the actual numbers of cases. Unfortunately the latter figures are not available. Although the mortality from the disease is small it seems not unreasonable to suppose, however, that it is, despite the opinion held by certain authorities, a fair index of the incidence of the disease.

When the incidence of rheumatic fever in different parts of England and Wales is investigated, it is found that the distribution by districts as indicated by the relative size of the death-rate is by no means uniform. The mortality is, as a rule, decidedly greater in the western and northern than in the eastern and midland counties. This is shown well in Table I which gives the standardised death-rates for rheumatic fever and rheumatism of the heart amongst persons 15 years and upwards for the respective counties of England and Wales in the decennium 1901-10. A similar excess of rheumatic fever is found in the western counties of the lowlands of Scotland, the death-rate reaching its maximum in the county of Renfrew as is shown in Table II. This excessive incidence does not obtain to the same degree, however, in the western counties of the Highlands of Scotland and is not a feature of a few of the counties in the west of England and in Wales. To these exceptions reference will be made later.

The meteorological factors of which the influence has been investigated are rainfall and temperature. The average annual rainfall for each several county has been estimated by superimposing the outlines of the areas with different amounts of rainfall as shown on the map of the British Isles prepared by Dr H. R. Mill, Director of the British Rainfall Organisation, on another map of large size showing the boundaries of the counties. From the latter map it has been possible by the aid of the planimeter to obtain the area of each county, the areas therein with different amounts of average annual rainfall and from these to calculate the average annual fall for the county. The figures for mean actual annual temperature for the respective counties have been obtained by a similar method from a map prepared by Prof. A. J. Herbertson and published in Bartholomew's Meteorological Atlas. In this map the actual temperature in different places is shown and not, as is usual, the temperature reduced to sea-level. It will be readily understood that the mean actual temperature calculated for a complete county from these different values can only be accepted as a fair criterion of the climate to which the majority of the people therein are subject if these are more or less evenly distributed throughout the county as is the case in many counties of England and Wales. It may be easily ascertained by reference to a map of Britain depicting the varying density of the population in persons per

Table I.

Registration County	Mean annual rainfall in inches	Mean actual annual temper- ature (°F)	Standardised death rate for rheu- matic fever and rheumatism of the heart from age 15 years and upwards, calculated from data for the period 1901-1910
London	27·5	49·8	0·0687
Surrey	27·3	49·5	0·0551
Kent	26·9	49·7	0·0624
Sussex	28·6	50·4	0·0682
Hampshire	33·5	49·5	0·0572
Berkshire	26·0	49·7	0·0618
Middlesex	27·5	49·8	0·0655
Hertfordshire	27·5	49·3	0·0660
Buckinghamshire	27·5	49·1	0·0555
Oxfordshire	27·5	48·9	0·0567
Northamptonshire	25·1	48·9	0·0698
Huntingdonshire	22·5	48·0	0·0553
Bedfordshire	25·1	48·1	0·0702
Cambridgeshire	22·5	48·9	0·0599
Essex	22·5	48·9	0·0684
Suffolk	24·2	48·9	0·0560
Norfolk	26·2	48·9	0·0513
Wiltshire	32·1	48·7	0·0545
Dorsetshire	35·0	50·6	0·0556
Devonshire	42·1	50·9	0·0606
Cornwall	47·1	50·3	0·0553
Somersetshire	34·2	49·6	0·0545
Gloucestershire	31·8	49·4	0·0625
Herefordshire	35·1	48·5	0·0694
Shropshire	29·9	48·5	0·0538
Staffordshire	30·9	48·1	0·0720
Worcestershire	27·5	48·8	0·0606
Warwickshire	27·5	48·8	0·0634
Leicestershire	27·5	48·8	0·0608
Rutlandshire	27·5	48·8	0·0693
Lincolnshire	24·7	48·6	0·0570
Nottinghamshire	24·1	48·9	0·0582
Derbyshire	36·1	46·1	0·0654
Cheshire	31·9	47·8	0·0778
Lancashire	41·8	47·2	0·1016
Yorkshire, West Riding	34·3	46·4	0·0862
„ East Riding	26·7	48·1	0·0621
„ North Riding	33·7	46·4	0·0853
Durham	32·5	45·7	0·0844
Northumberland	33·9	45·4	0·0778
Cumberland	41·9	46·4	0·0909
Westmorland	62·4	45·2	0·0878
Monmouthshire	35·8	49·3	0·0864
Glamorganshire	51·6	48·7	0·1067
Carmarthenshire	52·8	47·8	0·0914
Pembrokeshire	42·6	49·7	0·0752
Cardiganshire	48·2	47·3	0·0768
Brecknockshire	52·6	45·5	0·0996
Radnorshire	45·8	41·8	0·0514
Montgomeryshire	52·9	46·4	0·1035
Flintshire	31·6	48·6	0·0958
Denbighshire	46·8	47·2	0·0854
Merionethshire	65·3	46·1	0·1299
Carnarvonshire	47·9	47·2	0·0813
Anglesey	39·1	49·6	0·0495

Table II.

Counties or county groups in Scotland				Mean annual rainfall in inches	Mean actual annual tempera- ture (° F)	Standardised death- rate for rheumatism including rheumatic fever from age 0-45 calculated from data for the period 1881- 1900	Standardised death- rate for rheumatism including rheumatic fever from age 45 and upwards calculated from data for the period 1881-1900
1.	Sutherland	52.2	44.5	0.0656	0.3159
2.	Ross	42.8	44.7	0.0619	0.4033
3.	Inverness	53.1	44.3	0.0780	0.4157
4.	Argyll	60.0	46.0	0.0744	0.2651
5.	Perth, Kinross and Clackmannan	51.6	43.5	0.0704	0.2066
6.	Nairn, Elgin, Banff and Aberdeen	33.6	44.4	0.0719	0.1890
7.	Kincardine, Forfar and Fife	34.3	45.4	0.0592	0.2238
8.	Dumbarton and Stirling	59.9	45.6	0.0612	0.2159
9.	Linlithgow, Edinburgh, Haddington and Berwick	33.3	45.3	0.0746	0.2579
10.	Peebles, Selkirk and Roxburgh	43.6	43.3	0.0487	0.1740
11.	Ayr and Wigton	46.0	46.0	0.1281	0.4062
12.	Kirkeudbright and Dumfries	51.4	45.3	0.1261	0.4820
13.	Lanark	43.1	44.4	0.1251	0.4721
14.	Renfrew	55.6	45.6	0.1691	0.5987

square mile throughout the country that some of the counties of Scotland, especially those in the west Highlands, are to a very large extent uninhabited, the very scanty population being restricted to certain more or less well-defined areas largely coastal in their distribution. For these counties of Scotland, therefore, and to a less extent for others which have also very few inhabitants in considerable areas the mean actual temperature can thus obviously not be to the same degree as in England, a legitimate criterion of unfavourable climatic conditions and in this circumstance may rest in part at least the explanation of the non-observance in the case of the Scottish counties of the indirect relationship between mean actual temperature and rheumatic fever which is well-marked, as will be shown later, in the counties of England and Wales. The concentration of people in large towns will tend in a similar manner to render the mean actual temperature for the counties a less reliable measure of the climatic conditions to which the inhabitants are actually exposed. It is at the best, however, only an approximate measure and is used because, in an inquiry like this, data as far as possible independent of the personal equation of the observer are essential.

Considering in the first place the relationship between rainfall and the amount of rheumatism it is found that the remarks of previous observers are hardly borne out. The coefficient of correlation between the mean annual rainfall in the 55 counties of England and Wales and the standardised death-rate from rheumatic fever amongst persons from 15 years and upwards in the corresponding counties is very considerable, namely, + 0.683. Being positive and of such magnitude in comparison with its probable error it indicates that in the various counties of England and Wales for the decennial period 1901-10, there is a distinct tendency for excess in amount of rainfall to be associated with an increased death-rate from, and presumably an increased prevalence of, rheumatic fever. When the death-rates from rheumatic

fever in the several counties and the corresponding mean annual temperatures are compared in a similar manner the coefficient of correlation is found to be -0.538 , in this case negative in sign and also significant in value. From this the inference is that a reduced mean temperature has a distinct relationship to the incidence of the disease in the several counties and, since the correlation is inverse, the death-rate is higher and presumably the malady is more prevalent with an excess of cold weather throughout the year.

To differentiate the influence of rainfall from that of temperature the partial correlation coefficients between rainfall and temperature respectively and the standardised death-rate from rheumatic fever in the period 1901-10 have been calculated. It is found that when the temperature is considered to remain constant the coefficient between rainfall and rheumatic fever is $+0.579$ while when rainfall is considered constant the coefficient between mean temperature and rheumatism is -0.338 . This suggests that while excess in the amount of rainfall and the amount of cold weather have each a decided influence independently of the other, the excess of rainfall is the more potent factor. By means of a regression equation for rheumatic fever death-rate on mean annual rainfall and mean actual temperature theoretical values have been calculated which when compared with the actual death-rates for rheumatic fever in the counties of England and Wales for the period 1901-10 provide, on the whole, a moderately good fit. A few counties, notable amongst which are Cornwall and Anglesey, show actual values appreciably in defect of the theoretical and others, especially Lancashire and Glamorgan-shire, have death-rates distinctly in excess of what would be expected. An explanation of the former, and of a similar defect in the counties of the west Highlands of Scotland to be referred to later, may be found in their intimate and extensive relation to the western sea with consequent exposure to a relatively warm rainfall, less liable to cause chills which are so frequently the antecedents of acute rheumatism. In the latter counties, on the other hand, the high degree of urbanisation and unfavourable industrial conditions may, in addition to the meteorological, predispose to an excessive incidence of and death-rate from the disease.

In view of the differences above found it was suggested that in place of taking England and Wales as a whole, sub-division might furnish more definite information. The western counties of England, namely Cornwall, Devon, Somerset, Gloucester, Shropshire, Hereford, Cheshire, Lancashire, Westmorland, Cumberland and those of Wales, in all 23 in number, and the twelve counties on the east coast have been investigated separately. With regard to the first group the correlation between the rheumatic fever death-rate and the rainfall is found to be $+0.595$ while that between the temperature and the rheumatic fever death-rate is -0.477 . The coefficient between rainfall and temperature is -0.579 whence it has been calculated that the partial correlation between rainfall and the rheumatic fever death-rate is $+0.455$ and that between mean temperature and rheumatic fever -0.201 .

The coefficients for the eastern counties of England, are respectively $+0.821$ for rheumatic fever and rainfall, -0.756 for rheumatic fever and temperature and the partial correlation coefficients $+0.534$ between rheumatic fever and rainfall when temperature is constant and -0.244 between rheumatic fever and temperature when rainfall is considered constant. As all these coefficients except the last are more than three times their probable error the conclusion may be drawn that the relationship that obtains between rainfall and temperature respectively and rheumatic fever death-rate in the eastern and western groups of counties is very similar to that found to hold for the counties of England and Wales as a whole.

Proceeding now to consider the data for Scotland it is found that these are not so satisfactory to work with. In the supplements to the reports of the Registrar-General the returns for all varieties of rheumatic affections are gathered together under the heading, "rheumatism including rheumatic fever." It is found, however, that the deaths in the age period 0-45 years include the great majority of the deaths from rheumatic fever or acute rheumatism and exclude practically the whole of those from the more chronic conditions. This is shown by an analysis of the figures giving the number of deaths from rheumatic fever and closely allied conditions in Scotland in the different age-groups for the five years period 1901-05. In the annual reports for these years, rheumatic fever, rheumatism of the heart, chronic rheumatism, rheumatic arthritis, rheumatic gout and gout are tabulated separately. Of the total number of deaths from the four last-named diseases less than 10 per cent. occur under 45 years of age and these amount to only 4.2 per cent. of the total deaths in the "rheumatism" group. On the other hand, of the total deaths from rheumatic fever and rheumatism of the heart recorded for the same period, only about 28 per cent. occur after the age of 45 years. The standardised death-rate for rheumatism from age 0-45 years can thus be taken as a fairly reliable measure of the prevalence of rheumatic fever.

The counties of Scotland, as has been mentioned, have, for the purpose of the inquiry, been grouped in 14 groups, the small counties being associated with the adjacent larger counties and contiguous counties of relatively small extent or similar latitude taken together to obtain areas, which, while of convenient extent and furnishing a suitable number of cases are subject to more or less similar conditions. The groups are shown in Table II. The mean annual rainfall for the grouped county areas was first correlated with the standardised death-rate for rheumatism from age 0-45 years in the corresponding regions and the coefficient found was $+0.295$. This, while positive in sign like that for the counties of England, can scarcely be regarded as significant on account of its large probable error. When, however, the eastern and southern counties are alone considered the value of the coefficient is increased to $+0.480$. The correlation found between actual annual temperature and the death-rate for rheumatic fever in the Scottish counties is also insignificant when allowance is made for its probable error. This may,

however, be partly due, as has been explained, to the unsuitability of mean temperature as a criterion of actual exposure to climatic conditions in some of the counties of Scotland. It is of interest to note that the correlation between the rainfall values for the eastern and southern counties of Scotland and the standardised death-rate for rheumatism from age 45 and upwards for the same epoch, 1881-1900, is $+0.469$, which is very similar in value to that found for the age period 0-45 years. This suggests on the part of the cases providing the deaths in the two disease groups—the rheumatic fever and the rheumatoid—a very similar reaction to external or prevailing conditions. Support is given to this view by the circumstance that the correlation between the standardised death-rates for rheumatism in the age-groups 0-45 years and 45 years and upwards is $+0.906$. In regard to this it may be mentioned, in view of the contrast it presents, that the correlation coefficient between the standardised death-rates for rheumatic fever and rheumatism of the heart alone in the two age-groups 15-45 years and 45 years and upwards in the counties of England and Wales is $+0.319$, a value which suggests some lack of homogeneity in the nature of the cases that are recorded as terminating fatally from rheumatic fever at different ages.

The excessive death-rate from rheumatic fever generally found in the western districts as compared with the eastern which has already been alluded to, suggests the existence of conditions in the former specially favourable to the development and activity of the causal organism. The rheumatic fever death-rate has been shown to be highly correlated with the annual rainfall and when the regional distribution of the latter is examined it is found to exhibit a striking similarity to that of the former. There is the same contrast between the values for the western and those for the eastern counties. The excessive rainfall in the west is principally due to what has been termed purely geographical rain caused by a moist wind blowing from the west or south-west—the direction of the prevailing winds in Britain—against elevated land or mountainous ranges, the amount of the rain being largely determined by the contour of the land and its distance from the sea. With few exceptions of which the principal are Somerset, Gloucester, Hereford, Shropshire, Cheshire and Monmouth the counties in the west of England and in Wales have an average annual rainfall of over 40 inches while that in Westmorland, Glamorgan, Carmarthen, Brecknock, Montgomery and Merioneth exceeds 50 inches. The eastern counties, on the other hand, show, in the majority of instances, an average annual rainfall considerably less in amount and as a rule under 30 inches, the only counties which slightly exceed this figure being Northumberland, Durham and the North Riding of Yorkshire. The eastern counties of Scotland are likewise subject to considerably less rainfall during the year than those further west. The closely analogous distribution of excessive rainfall and excessive incidence of rheumatic fever in the counties of England and Wales described above indicates very strongly that of the meteorological factors which have been investigated excessive rainfall exerts

Table III.

Coefficients of correlation.

Variables	<i>r</i>
Standardised death-rate for rheumatic fever for persons from age 15 years and upwards and mean annual rainfall. (55 counties of England and Wales 1901-1910)	+0.683 ± 0.049
Standardised death-rate for rheumatic fever for persons from age 15 years and upwards and mean annual rainfall. (23 western counties of England and Wales 1901-1910)	+0.595 ± 0.091
Standardised death-rate for rheumatic fever for persons from age 15 years and upwards and mean annual rainfall. (12 eastern counties of England 1901-1910)	+0.821 ± 0.063
Standardised death-rate for rheumatic fever for persons from age 15 years and upwards and mean actual annual temperature. (55 counties of England and Wales 1901-1910)	-0.538 ± 0.065
Standardised death-rate for rheumatic fever for persons from age 15 years and upwards and mean actual annual temperature. (23 western counties of England and Wales 1901-1910)	-0.477 ± 0.109
Standardised death-rate for rheumatic fever for persons from age 15 years and upwards and mean actual annual temperature. (12 eastern counties of England 1901-1910)	-0.756 ± 0.083

Table IV.

Partial correlation coefficients.

Variables	<i>r</i>
Rheumatic fever death-rate and rainfall with temperature constant. (55 counties, England and Wales, 1901-1910)	+0.579 ± 0.060
Rheumatic fever death-rate and rainfall with temperature constant. (23 western counties, England and Wales 1901-1910)	+0.445 ± 0.113
Rheumatic fever death-rate and rainfall with temperature constant. (12 eastern counties, England 1901-1910)	+0.534 ± 0.139
Rheumatic fever death-rate and temperature with rainfall constant. (55 counties, England and Wales 1901-1910)	-0.338 ± 0.081
Rheumatic fever death-rate and temperature with rainfall constant. (23 western counties, England and Wales 1901-1910)	-0.201 ± 0.135
Rheumatic fever death-rate and temperature with rainfall constant. (12 eastern counties, England 1901-1910)	-0.244 ± 0.183

a predominant influence on the prevalence of the disease. While the mean actual temperature may not be in some districts, for the reasons already given, a sufficiently reliable criterion of unfavourable climatic conditions, the relatively high inverse correlation found in the counties of England and Wales between it and the death-rate for rheumatic fever undoubtedly suggests that it also is of considerable importance as a causal factor and that an excessive amount of cold weather is closely associated with an excessive incidence of the disease.

As these conclusions indicate divergence from prevalent views they seem to suggest the need for further investigation into some of what are more or less generally regarded as important amongst the predisposing causes of rheumatic fever, a disease of special interest on account of the frequency and seriousness of the complications liable to follow its onset.

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TRENCH FEVER.

FINAL REPORT OF THE WAR OFFICE TRENCH FEVER
INVESTIGATION COMMITTEE.

EDITED BY

MAJOR GENERAL SIR DAVID BRUCE, A.M.S.
CHAIRMAN.

CONTENTS.

	PAGE
INTRODUCTION	259
I. IN WHAT TISSUES OF THE BLOOD IS THE ORGANISM OF TRENCH FEVER FOUND? .	259
1. Whole blood	263
II. THE RELATION OF THE ORGANISM TO THE BLOOD	263
1. Blood plasma	264
2. Blood serum	264
3. Red blood corpuscles	264
4. Laked whole blood	265
5. Minimal quantity of whole blood required	265
6. Inoculation of scarified area of skin by whole blood	266
7. When does the blood become infective?	266
8. How long does the blood remain infective?	267
9. Microscopical examination of the blood	267
III. HOW DOES THE ORGANISM LEAVE THE BODY?	267
1. In the sputum	268
2. In the urine	268
3. In the faeces	268
IV. HOW DOES THE ORGANISM ENTER THE BODY?	269
1. By the mouth or nasal passages	269
2. Through the conjunctiva and other mucous membranes	269
V. THE LOUSE AS THE CARRIER OF THE TRENCH FEVER ORGANISM	270
1. Is the organism transferred to man through the proboscis of the louse? .	270
2. By the excreta of infected lice?	271
3. By the excreta of clean lice?	271
4. By the feeding of clean lice through a layer of infected excreta? . . .	272
5. By the crushing of bodies of infected lice?	272
6. Do lice fed on a case within 12 hours of onset of disease become infective?	272
7. Do lice fed on an afebrile case become infective?	273
8. At what temperature do lice require to be kept between feedings? .	273
9. What time elapses between an infected feed and the passage of infective louse excreta?	273 274
10. What percentage of lice become infective?	274
11. Hereditary transmission of the organism in the louse	275
12. Does <i>Pediculus capitis</i> convey the organism?	275
13. The minimal infecting dose of louse excreta	275
VI. VIABILITY OF THE TRENCH FEVER ORGANISM	276
1. Effect of drying on the blood	276
2. Exposure to sunlight	276

	PAGE
3. Effect of distilled water	276
4. How long do louse excreta remain infective?	277
5. At what temperature is the organism killed: (a) by dry heat, (b) by moist heat?	277
6. Effect of ultra-violet rays on the organism	278
7. Effect of antiseptics and other chemical substances	278
VII. ARE ANY OF THE LABORATORY ANIMALS SUSCEPTIBLE TO TRENCH FEVER	279
1. The monkey	279
2. The rabbit and guinea-pig	279
VIII. IMMUNITY	279
1. Are some men naturally immune?	279
2. Does an attack of the disease confer immunity?	280
IX. ETIOLOGY	283
1. <i>Rickettsia</i> -like organisms found in the excreta of infected lice	283
2. Are lice showing <i>Rickettsia</i> infective?	283
3. How early in the disease can a patient cause lice fed on him to show <i>Rickettsia</i> ?	284
X. TO ASCERTAIN IF THE TRENCH FEVER ORGANISM CAN BE TRANSMITTED BY OTHER BLOOD-SUCKING INSECTS	284
1. By the bug	284
XI. CULTIVATION OF THE TRENCH FEVER ORGANISM IN ARTIFICIAL MEDIA	285
XII. FILTERABILITY OF THE ORGANISM	285
1. When present in the blood	285
2. In the excreta of infected lice	286

INTRODUCTION.

THE chief object of this report is to place on record the remainder of the experimental work carried out under the direction of the War Office Trench Fever Investigation Committee by Major Byam and his co-workers at the New End Military Hospital, Hampstead, during part of 1917 and the two following years.

This Committee was formed in November 1917 by Lieut.-General Sir ALFRED KEOGH, G.C.B., the Director General of the Army Medical Services, for the purpose of investigating Trench Fever with a view to the discovery of its causation, mode of spread and prevention.

The Committee consisted of:

Major General Sir DAVID BRUCE, K.C.B., F.R.S., A.M.S., Chairman.

J. A. ARKWRIGHT, Esq., M.A., M.D., D.Sc., Lister Institute.

A. W. BACOT, Esq., F.E.S., Lister Institute.

J. E. BARNARD, Esq., P.R.M.S.

Major W. BYAM, R.A.M.C.

Professor JOHN EYRE, M.D.

Sir W. M. FLETCHER, K.B.E., F.R.S.

Lieut.-Colonel H. FRENCH, C.B.E., R.A.M.C. (Temp.)

Lieut.-Colonel D. HARVEY, C.M.G., R.A.M.C.

The late Professor H. PLIMMER, F.R.S. and Lieut. A. F. HIRD, General List (Temp.), Secretary.

Lieut.-Colonel LEDINGHAM, C.M.G., F.R.S., Lister Institute, after his return from active service, also served on the Committee.

The Committee held their first meeting on the 12th Nov. 1917, when Major Byam stated that he already had a number of officers working on this subject under his direction at the Hampstead Military Hospital.

The names of the working party were:

Major W. BYAM, R.A.M.C.

Captain J. H. CARROLL, U.S.R.

Captain J. H. CHURCHILL, R.A.M.C. (T.)

Captain LYN DIMOND, R.A.M.C.

Captain V. E. SORAPURE, R.A.M.C.

Captain R. M. WILSON, R.A.M.C.

Captain PEACOCK, R.A.M.C. (T.), Entomologist.

Lieut. LL. LLOYD, R.A.M.C. (T.) was appointed at the beginning of 1918 in

Captain Peacock's place on the latter's transfer to France.

In addition to the working party at Hampstead, Messrs Arkwright, Bacot, and Ledingham of the Lister Institute, members of the War Office Committee, took a prominent part in the research, especially in regard to the part played by the louse in the spread of the disease and the relation of the so-called *Rickettsia*-bodies to Trench Fever.

Mr J. E. Barnard, another member of the Committee, also gave conspicuous aid to the working party in the microscopic examination and filtration of the blood and the action of ultra-violet rays on the virus.

Lieut.-Colonel French deserves honorary mention as it was by his instrumentality that the first volunteers were found. Without the volunteers the main object of the work could not have been attained.

The War Office Committee met as a rule at weekly intervals. Its last meeting, the 83rd, was held on January 19th, 1920, when Colonel D. Harvey, the acting chairman, announced that the Army Council had dissolved the Committee.

Major Byam and his co-workers have already described the larger part of the results of their work; first in a paper read before the Royal Society of Tropical Medicine and Hygiene on May 17th, 1918, and later in book form, entitled *Trench Fever*, Oxford Medical Publications, which appeared in 1919.

Lieut. Lloyd the entomologist also brought out a book, called *Lice and their Menace to Man* in the same year, and by the same publishers, which included some of the work done at Hampstead, especially in the chapter on the migration of body lice from trench fever cases to healthy men.

Other publications have also appeared which arose out of this work: *The Nervous Heart*, by Captains R. M. Wilson and John H. Carroll; "The Association of *Rickettsia* with Trench Fever," by J. A. Arkwright, A. Bacot and F. Martin Duncan, *Journal of Hygiene*, vol. xviii. no. 1, April 15, 1919; "Agglutination Experiments with Trench Fever *Rickettsia*," by J. C. G. Ledingham, *The Lancet*, vol. i. p. 1264, 1920.

About the same time that this investigation was being begun in England, the American Red Cross were organising a similar research in France.

In October 1917 at the first meeting of the Medical Research Committee of the American Red Cross in Paris, Major R. P. Strong recommended that a research into Trench Fever should be undertaken. He stated that after several months' study of the problems relating to the prevention of infectious diseases occurring in the Allied Armies on the Western Front it became evident that the subject of the method of transmission of Trench Fever was one of the most important for investigation in connection with the loss of man-power in the fighting forces.

At the next meeting in November 1917 this was agreed to, and a Trench Fever Committee under the chairmanship of Major Strong was formed. In co-operation with the Medical Investigation Committee of the British Expeditionary Force the research was organised and experiments begun on February 4th, 1918. The members of this Commission were eight in number, seven Americans and one Englishman, Lieut. A. D. Peacock, R.A.M.C., as entomologist. In less than six months the investigation was completed and the Report in the hands of the printers. This is entitled, *Trench Fever Report of Commission, Medical Research Committee, American Red Cross*, Oxford University Press, 1918, and was edited by Major Strong. This report represents an extraordinary able piece of research, and shows the great advantage in rapidity to be gained from team work.

It may be considered that there was now no necessity for the War Office Trench Fever Committee to continue its work in London. It must be remembered however that Major Byam and his team had been working at the subject for some time before the War Office Committee was formed, also that it was important that the work should be confirmed in England at a distance from the front line in France where cases of the disease were numerous. Further as all the experimental work had to be performed on man, the number was necessarily limited and controls inadequate. The work of one Commission would often therefore serve to supplement the work of the other and lessen to some extent these disabilities. The result of the work of the two Commissions justifies this, as the conclusions arrived at are by no means identical. For example the American Commission came to the conclusion that the organism causing the disease is a resistant filterable virus; whereas the British concluded that it was neither filterable nor ultra-microscopic, which the word filterable is usually supposed to imply, but was in fact a species of *Rickettsia* related to the organism found in typhus fever. Again the American Commission states that the usual manner of infection is by the bite of the louse. The British Commission on the other hand is of opinion that infection by the bite is quite exceptional and that by far the commonest method is by infection of scratches or other small wounds, with the excreta of the louse.

Moreover the American Commission had for their main object the demonstration and experimental proof of the mode of transmission of the disease, for the practical purpose of preventing the loss of man-power at the front; the object of the English Committee was to make a complete investigation

of the disease from every point of view; and it is to be regretted that the exigencies of the Service caused the Army Council to close down the Committee before this was by any means accomplished.

It may be thought that as Trench Fever disappeared at the conclusion of the war, it is not necessary to place these remaining experiments on record. On the other hand it would appear to be all the more important since there will probably be no further opportunity of studying the disease until the next European war. Again as most of these experiments were carried out on man himself, and a good deal of expense incurred, it would appear to be unwise to allow any of the knowledge gained to lapse.

This Report then is merely meant to place on record the remaining experiments carried out at the Trench Fever Hospital, Hampstead, by Major Byam, and his colleagues. No attempt will be made to describe the clinical features, geographical distribution, pathology, diagnosis or treatment of the disease. These are all dealt with in the reports mentioned above, and in numerous other papers and need not be repeated here.

It may be recorded here that several of the staff at Hampstead became infected with Trench Fever in the course of the investigation. Captain Carroll, one of the medical officers, and Sister Pullen, one of the nursing staff, became infected, but the exact method of infection was not ascertained.

Sergeants Clifford and Heylock, laboratory assistants, were also attacked by the disease; both frequently handled the infected boxes of lice and collected the louse excreta.

Dr Arkwright and Mr Bacot of the Lister Institute also became infected, probably through the bites or excreta of infected lice.

In conclusion the Committee would wish to express their most sincere thanks and admiration for the excellent work done by Major Byam and his team of workers at Hampstead. They worked without sparing themselves from the beginning to the end of the investigation with the greatest zeal, intelligence and enthusiasm and have made a notable advance in our knowledge of the infectious diseases.

The Committee would also desire to convey their heartiest thanks to the nursing staff of New End Hospital. The Sisters took the greatest possible interest in the investigation and aided and co-operated with the medical workers in every possible way.

Without the volunteers nothing could have been achieved. These men, unable through age or other infirmities to go to the front, bravely did their bit for the sake of their comrades in the trenches, by allowing themselves to be inoculated with an often tedious and painful disease. The Army owes them the deepest gratitude.

The Committee would also beg to acknowledge the constant assistance and encouragement they received from Lieut.-General Sir Alfred Keogh, G.C.B., Director General of the Army Medical Services and his successor Lieut.-General Sir T. H. Goodwin, K.C.B.

The Committee also beg to place on record the generosity of the Lister Institute of Preventive Medicine. Not only did this Institute provide several workers to help in the investigation, but it also financed the experimental work to the extent of £2300. Without this assistance it is difficult to know how the work could have been carried out.

As it will not be possible on account of expense, to print the individual experiments in full, we beg to inform future workers on Trench Fever, that all the original papers, temperature charts, case sheets, etc., in connection with this investigation are preserved at the Lister Institute, Chelsea Gardens, London, and may be consulted there.

1. IN WHAT TISSUES OF THE BODY IS THE ORGANISM OF TRENCH FEVER FOUND?

1. *Whole blood.*

In the investigation of a disease one of the first things is to find out if there is any infecting organism present. This was first done by English workers in the case of Trench Fever by McNee, Brunt and Renshaw at the beginning of 1916. They succeeded in transferring the disease to healthy men by the injection of the blood of Trench Fever cases. This observation was confirmed by the American Commission in 1918. McNee and his colleagues carried out seven experiments with whole blood. These were all positive. The Americans had twelve positive and one negative result. The following table gives the experiments also made by the War Office Committee to confirm McNee's results.

Table I.

Exp. no.	Amount of blood injected in c.c.	Day of disease	Treatment of blood	Interval, in minutes, between collection and experiment	Route	Result
3	5	24	Citrated	8	Vein	+
4	5	9	"	5	Vein	+
7	5	3	"	$\frac{1}{2}$	Intra-muscular	-
15	5	2	"	1	Vein	+
19	5	51	"	1	Intra-muscular	+
36	5	2	"	1	Vein	+
135	5	1	"	1	Vein	+
139	5	1	"	3	Subcutaneous	-
174	10	5	"	2	Intra-muscular	-

In the above nine experiments it is seen that six are positive and three negative. On examining the protocols of the three negative experiments none of them are found to be satisfactory. The blood used in No. 139 was taken from a very mild case, on the first day of the attack: that used in No. 174 was from a case whose blood gave negative results in three experiments and for some unknown reason the blood seems to have been non-infective on the day of withdrawal. It will be noted that the three negative experiments follow on intra-muscular or subcutaneous injections; all those injected intravenously are positive.

Conclusion. It is abundantly proved that the Trench Fever organism is present in the "whole blood" of Trench Fever cases and that therefore it will be found in all the tissues of the body.

II. THE RELATION OF THE ORGANISM TO THE BLOOD.

1. *Blood plasma.*

After it was proved that the whole blood of Trench Fever patients contains the organism, experiments were made to discover in what part of the blood—plasma or corpuscles—the organism was located, in other words is the parasite intra-corpuseular or extra-corpuseular.

Table II.

Exp. no.	Amount of blood injected in c.c.	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
203	11	6	Citrated and centrifuged	3 hours 51 mins.	Vein	+
215	5	4	Citrated	8 hours	Vein	-

In the first place it was attempted to remove the corpuscles from the citrated blood by the centrifuge, and so to separate out the blood plasma.

McNee reports one such experiment with blood plasma which gave a positive result.

The American Commission made five experiments on similar lines which were all successful.

Only two experiments were made by the War Office Trench Fever Committee, the first was positive, the second negative. The second was done as a control to a filtration experiment and consisted in the withdrawal of 60 c.c. of blood from a Trench Fever patient. This blood was added to 300 c.c. citrate solution and allowed to sediment by gravity. 5 c.c. of the upper layer of the diluted plasma was pipetted off and injected. This is a very doubtful experiment and may be ignored.

Conclusion. In seven experiments with unfiltered plasma the result was positive. It may therefore be concluded that probably the organism of Trench Fever is to be found free in the fluid part of the blood, that it is extra-corpuseular and not intra-corpuseular.

2. *Blood serum.*

Table III.

Exp. no.	Amount of serum injected	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
16	4.5 c.c.	2	Clotted and centrifuged	3 hours and 25 min.	Vein	-

It would appear that when the blood is allowed to clot the micro-organisms of Trench Fever are entangled in the fibrin and so disappear from the clear serum which is separated.

McNee and his colleagues made two experiments with serum one of which was negative and one positive. The serum in the positive case was haemoglobin tinted, this condition being due according to McNee to faulty technique.

The American Commission also made an experiment with serum and failed to transmit the disease.

The one experiment made by the War Office Committee is given above.

Conclusion. When the blood of Trench Fever cases was allowed to clot naturally the separated serum in three cases was found to be sterile. Hence it may be inferred that when blood is allowed to clot the Trench Fever organisms are entangled in the fibrin and do not appear in the clear serum. Another conclusion might be that the virus is contained in the corpuscles and so disappears from the serum.

3. *Blood corpuscles.*

No attempt was made by the War Office Committee to ascertain if the micro-organism of Trench Fever is an intra-corpuscular parasite.

McNee, Renshaw and Brunt report two experiments with washed corpuscles; one negative and one positive. They are of opinion that their results point to the virus being contained within the blood corpuscles themselves; whether leucocytes or red cells.

The American Commission states that of four volunteers injected with washed blood cells, three developed the disease. The average incubation period was considerably prolonged over that obtained with the injection of whole plasma from the same cases and this they consider suggests that the virus was not in the blood cells, but that it existed mixed with them and had not been completely separated by the three washings.

Conclusion. The evidence on the whole appears to be more in favour of the virus being extra-corpuscular than intra-corpuscular, but it must be confessed that the proof of this is not very satisfactory. If the organism could only be demonstrated in the blood by means of the microscope this question would be cleared up, but this has not been done up to the present.

4. *Laked whole blood.*

Table IV.

Exp. no.	Amount of blood injected c.c.	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
5	2.7	9	10 c.c. blood added to 10 c.c. citrate of soda and then added to 170 c.c. of Aq. Dis. 20 c.c. of N.S. 10 times normal strength added to above	1½ hours	Vein	-
136	5	1	Laked in 20 c.c. Aq. Dis.	17 mins.	Vein	+

Experiments on the effect of laking infective Trench Fever blood were made in connection with filtration experiments. In the case of Exp. 5 it would appear that the addition of a large quantity of distilled water for the purpose of laking the blood had rendered the blood non-infective. That the blood was originally infective is shown in Exp. 4. In the second experiment when 5 c.c. of blood was laked by a smaller quantity of distilled water and kept for a shorter time the result was positive.

The number of experiments is too small to justify any conclusion.

5. *Minimal quantity of whole blood required.*

Table V.

Exp. no.	Amount of blood injected c.c.	Day of disease	Treatment of blood	Interval between collection and experiment mins.	Route	Result
87	0.5	1	Citrated	1	Vein	+
124	0.1	1	Citrated	1	Vein	-
173	0.1	5	Citrated	2	Vein	-

The last experiment (No. 173) is not satisfactory. When tested later for natural immunity the case also failed to react, although a large dose of virus was used.

From the above experiments it would appear that 0.5 c.c. of blood in one instance was sufficient to transfer Trench Fever to a healthy man, but that 0.1 c.c. was not enough.

It has been shown that the intra-venous method of transferring the blood from cases of Trench Fever to healthy men seems to be more successful than the intra-muscular or subcutaneous. An attempt was made to infect by inoculation of infective blood into a scarified area of the skin.

6. *Inoculation of scarified area of skin with whole blood.*

Table VI.

Exp. no.	Amount of blood used c.c.	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
38	10	2	None	1 min.	Skin	-
44	1	2	Dried at room temperature	8 days	Skin	-
88	0.5	1	Citrated	1 min.	Skin	-

In the above three experiments the attempt to transfer Trench Fever by the application of whole infected blood to the scarified skin was unsuccessful.

In Exps. 38 and 88 the blood was proved to be infective by other experiments carried out at the same time.

It will appear a plausible deduction from these experiments that either the organism of Trench Fever is very few and far between in infective blood, or the conditions are such as to render it less easily absorbed through the scarified skin. It will be found later that a very small quantity of infective

louse excreta rubbed into a scarified area of the skin almost always gives rise to the disease.

7. *To ascertain when blood of trench fever cases becomes infective.*

Table VII.

Exp. no.	Amount of blood injected	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
188	1 c.c.	Under 12 hrs.	Citrated	At once	Vein	—

Only one experiment was made with blood taken five hours after the first rise of temperature. From other evidence it would appear that the micro-organisms of Trench Fever are very scarce in the peripheral blood during the first twelve hours of the onset of the fever.

8. *How long does the blood of trench fever patients remain infective?*

Table VIII.

Exp. no.	Nature of experiment	Day of disease	Inoculation	Route	Result
72	Lice fed on old chronic case	298	Excreta rubbed into scarified area of skin	Skin	+
92	"	298	"	Skin	—
93	"	116	"	Skin	—
181	"	443	40 mg. excreta in 4 c.c. N.S.	Subcut.	+
184	"	182	8 mg. excreta in 1 c.c. N.S.	Subcut.	—
212	"	175	10 mg. excreta in 5 c.c. N.S.	Subcut.	—

This is important from the point of view of the spread of the infection. No experiments were made to answer the question by the direct injection of blood from old chronic cases into healthy volunteers. The method used was to feed lice on the old cases and afterwards inoculate the excreta of these lice into healthy individuals. This was found to be a much more certain method of setting up the disease than by the direct transference of blood, due probably to the multiplication of the virus in the intestine of the louse.

From the above six experiments it will be seen that cases of Trench Fever may retain the power of infecting lice fed on them up to at least 443 days after the onset of the disease. That all cases do not retain this infectivity is evident from the fact that four of the six experiments are negative.

9. *Microscopical examination of the blood.*

It may be said at the outset that up to the present no organism has been found in the blood, organs, urine or faeces of cases of Trench Fever which can undoubtedly be considered to be the cause of the disease. It is unnecessary to give an account here of the various organisms which have been described, as a summary has already been given by the American Red Cross Commission in their Report.

Mr Barnard reported to the War Office Committee that the examination of trench fever blood under dark ground illumination showed the presence of

minute granules which he had been unable to find in normal blood. These granules fluctuated in number from day to day reaching a maximum shortly before the rise in temperature. He considered these particles to measure about 0.1 micron in size and therefore to be much smaller than the *Rickettsia*-bodies found in the louse. This difference in apparent size may possibly have been due to the difference in mode of preparation; unstained and floating in a layer of blood in the one case, dried and stained in the other.

Mr Bacot states that, in the section of the American Red Cross Committee's Report dealing with the absence of visible micro-organisms in the blood and plasma of patients, it is admitted that a few bodies resembling the diplobacilli described by the German investigators as the cause of the disease were occasionally encountered in stained specimens of the blood and plasma but the report goes on to state that the American workers could obtain no definite evidence that these bore any etiological relationship to the disease.

Bacot adds that subsequent work in England and on the Continent has resulted in a fairly general acceptance of the fact that the presence of such bodies (*Rickettsia*), found in infected lice, is related to the power of the insect to convey the disease. The difficulty, however, of diagnosing the disease from the presence of a few such forms in blood films has not been surmounted because the scarcity of the bodies renders the confusion of artefacts of similar appearance possible.

Though workers on the Continent appear to be satisfied that they can use this method of diagnosing the disease with some certainty, American and English workers have up to the present been unable to accept such evidence with any confidence.

III. HOW DOES THE ORGANISM OF TRENCH FEVER LEAVE THE BODY?

1. *In the sputum and saliva.*

No experiments were made in England on this point. Three experiments to determine the infectivity of the sputum and saliva were made by the American Commission. The saliva and sputum were centrifuged and the sediment dried at 30° C. The sediment was then rubbed into scarified areas of the skin of three volunteers. Two of the experiments remained negative; one was positive.

The American Commission concluded that the sputum with the saliva mixed in it is sometimes infective.

It appears to us that the experiments are too few in number to justify any conclusion being drawn.

2. *In the urine.*

It would be surprising if the micro-organism of Trench Fever did not find its way out of the blood into the urine at some period or other of the disease. The three experiments made by the English workers are all negative. The

urine was taken at three different periods as will be seen from the following Table.

Table IX.

Exp. no.	Amount of urine	Day of disease	Treatment of urine	Interval between collection and experiment	Route	Result
69	30 c.c. morning and evening daily	19 to 25	Centrifuged and deposit dried at 37° C.	36 hours	Skin	—
78	30 c.c. morning and evening daily	1 to 10	Centrifuged and deposit dried at 37° C.	3 days	Skin	—
159	20 c.c. daily from two patients	11 to 20	Centrifuged and deposit dried at 37° C.	12 days	Skin	—

In all three cases the urine was centrifuged and dried at 37° C.

The American Commission were more successful. Eight experiments were made to determine the infectivity of the urine, of these five were positive.

Conclusion. From the results of the American Commission's experiments it may be concluded that the urine of Trench Fever cases does frequently contain the organism of the disease. It is curious that none of the English experiments showed a positive result.

3. *In the faeces.*

No experiments were made by the English Committee.

Three experiments were carried out by the American Commission. A portion of the faeces taken from Trench Fever cases was smeared in thin layers in Petri dishes and dried in the open air; all the specimens were mixed and ground in a mortar. A small amount of the resulting powder was then moistened with normal saline solution and made into a paste. This was then rubbed into scarified areas of the skin of the volunteers. All the experiments were negative.

The American Commission conclude from these experiments that there is no evidence that the faeces are infective in Trench Fever.

It will be shown later that infection probably does not take place by way of the mouth in food or drink, or by way of the mucous membrane of the nose. If this is true then the importance of the infectivity of the saliva, sputum, urine or faeces is robbed of much of its significance.

IV. HOW DOES THE ORGANISM OF TRENCH FEVER ENTER THE BODY?

1. *By the mouth or nasal passages.*

Table X.

Exp. no.	Nature of experiment	No. of feeds	Route	Result
13	Excreta of lice given by the mouth and by inhalation	15	Mouth and nose	—
14	" " "	9	Mouth and nose	—
120	Only by the mouth	6	Mouth	—

In these few experiments large quantities of infective material were given by the mouth and nose with negative results. From this it may be concluded

that the contamination of food by the urine, sputum or saliva of affected persons or by the excreta of infected lice does not constitute a danger.

In other words the virus of Trench Fever is not conveyed from the sick to the healthy by way of the mouth in eating or by way of the nose in breathing.

2. *Through the conjunctiva and other mucous membranes.*

Table XI.

Exp. no.	Amount of excreta used	No. of inoculations	Age of excreta	Route	Result
77	From 500 lice	3	24 days	Conjunctiva	+
119	From 400 lice	1	15 days	„	+
205		1	96 days	Urethra	-
207	10 mgm.	3	93 days	„	-

It is curious that the sniffing up the nose of the infected excreta of lice did not give rise to infection, whereas the placing of the same material in the conjunctival sac did give rise to positive results in two cases.

The attempt to infect by way of the urethra was unsuccessful.

It may be concluded that the micro-organism of Trench Fever does not enter the body by way of the mouth in the act of eating or drinking, by the nose in breathing, or by the contact of infective material with healthy skin. It would appear then to be probable that, as it is found constantly in the blood, the mode of infection will be by a blood sucking insect.

V. THE LOUSE AS THE CARRIER OF THE TRENCH FEVER ORGANISM.

That the louse is the carrier of the organism of Trench Fever has now been abundantly proved. It is unnecessary to repeat the history of this discovery since it is given by Major Byam and his colleagues in their report, and by the American Red Cross Commission. Suffice it to say that as early as July 1916 Hunt and McNee write that they think sufficient evidence has now been obtained that the body louse is the parasite concerned although absolute proof is lacking, as they had been unable to carry out actual transmission experiments.

To the War Office Trench Fever Committee is due the honour of having been the first to demonstrate by experiment the part played by the louse in the transmission of Trench Fever, the first successful result having been obtained on the 14th Feb., 1918. On the 9th March the American Commission also announced positive results.

There is some doubt as to whether the disease is transferred from the louse to man in the act of biting, or by the inoculation of the excreta of the louse into abraded surfaces. Table XII shows the results following the bite of the louse.

From a consideration of Tables XII-XIV it is manifest that infection through the proboscis of the louse in the act of biting rarely happens and only after a long time. The incubation period in the four cases which gave a positive

1. *Is the organism transferred through the proboscis of the louse?*

Table XII.

Exp. no.	No. of days over which feeding extended	Number of bites estimated	Incubation in days	Result	Remarks
1	35	9,518	...	-	
2	41	13,224	...	-	
40	17	4,945	...	-	
47	14	23,821	...	-	Lice let loose excreta not removed
48	14	25,609	14	+	Fed through chiffon, excreta wiped off with cotton wool
50	14	21,908	...	-	
67	5	7,520	...	-	Skin washed with abs. alcohol after feed
68	32	40,580	35	+	Skin washed with abs. alcohol after feed
149	26	-	Skin washed lysol no scratching
176	18	14,600	18	+	Skin washed lysol
188	43	6,136	44	+	Fed through chiffon, washed lysol; scratched himself

2. *By the excreta of infected lice.*

Table XIII.

Exp. no.	Amount of excreta	Day of disease	Treatment of excreta	Interval between collection and experiment	Route	Result
8	576 lice	Various	None	6 hours	Skin	+
10	...	"	"	15 minutes	"	+
11	...	"	"	"	"	+
12	...	"	"	1 hour and 15 mins.	"	+
32	...	"	"	3 hours	"	+
56	...	8 to 16	"	27 days	"	+
64	...	27 to 28	"	1 hour	"	+
72	300 lice	298 to 339	"	1 day	"	+
81	500 lice	27 to 28	"	...	"	+
92	300 lice	298 to 339	"	21 days	"	-
93	250 lice	116 to 118	"	19 "	"	-
96	2 mgm.	9	In 1 c.c. N.S.	...	Subcut.	+
97	0.1 mgm.	9	"	...	"	+
102	0.05 "	9	"	...	"	-
109	0.1 "	9	"	...	"	-
127	1 "	2 to 25	"	29 days	"	+
130	2 "	9	"	70 "	"	-
138	8 "	3 to 14	In 1.5 c.c. N.S.	86 "	"	+
148	8 "	Various	In 1 c.c. N.S.	1 to 74 days	"	+
152	40 "	"	" 4 "	5 and more	"	+
172	5 "	"	" 2 "	...	"	+
182	1 "	16 to 18	" 1 "	39 days	"	+
198	10 "	Various	" 2 "	84 "	"	+
208	...	"	None	130 "	"	+
214	8 "	"	In 1 c.c. N.S.	...	"	+

3. *By the excreta of clean lice.*

Table XIV.

Exp. no.	Amount of excreta	Treatment of excreta	Interval between collection and experiment	Route	Result
17	300 lice	None	17 hours	Scarified skin	-
18	"	"	"	"	-
213	...	"	48 "	"	-

result averaged 28 days. It is possible that this mode of infection never takes place at all as it must be difficult altogether to prevent the risk of infection through the skin by the excreta.

This is what would be expected as there is no evidence that the salivary glands of the louse are invaded as in malaria or trypanosomiasis and the danger of regurgitation of the intestinal contents into the proboscis seems remote.

On the other hand Table XIII shows how deadly a means of infection is provided by the excreta when brought in contact with an abraded surface of the skin. Practically in every case the disease is produced with the short average incubation period of 7·7 days.

Table XIV shows three control experiments with the excreta of clean lice; as will be seen all were negative.

4. *By the feeding of clean lice through a layer of infected excreta.*

It was thought by Major Byam and his colleagues that the small punctures made by the lice in the act of feeding might provide an entrance for the organism. Exp. 133 was devised to put this to the proof. Clean lice were made to feed on an area of skin covered with a thin moist layer of infected excreta. This was repeated on three occasions, but the result was negative.

5. *To ascertain if the virus of trench fever can be transferred from louse to man by crushing the bodies of infected lice on an area of scarified skin.*

Table XV.

Exp. no.	No. of lice used	Day of disease	Treatment of lice	Route	Result
9	11	11-23	Crushed	Scarified skin	+
156	1	2-11	Emulsified in N.S.	"	-
157	1	2-11	"	"	+
166	1	...	"	"	-
167	1	...	"	"	+

It is evident that in the scratching and rubbing which accompanies lousiness, lice must often be crushed and broken up. From the above experiments it is seen that on two occasions a single louse broken up in a drop of normal saline solution was capable of giving rise to the disease.

6. *To ascertain if lice fed on a trench fever case within 12 hours of the onset of the disease will become infective.*

Table XVI.

Exp. no.	Amount of excreta	Day of disease	Treatment of excreta	Interval between collection and experiment	Route	Result	Remarks
144	70 lice	First	None	12-18 days	Scarified skin	-	Lice fed once within 12 hrs. of rise of temp.
183	80 mgm. 500 lice	"	1.5 c.c. N.S.	70 days	"	+	Lice fed once within 12 hrs. of rise of temp.
191	20 mgm. 1000 lice	"	"	8-13 days	Subcut.	-	Lice fed once within 12 hrs. of rise of temp.

It will be remembered that in a former experiment (No. 188) 1 c.cm. of blood taken within five hours after the onset of the fever failed to give rise to the disease.

In the above three experiments lice were fed once within 12 hours after the onset. Afterwards their excreta was used for inoculation into healthy volunteers with one positive and two negative results.

It is probable then that the microorganisms of Trench Fever are present in the blood although in few numbers, at the beginning of the fever.

7. *To ascertain if lice fed during an afebrile stage become infective.*

Table XVII.

Exp. no.	Lice fed on	Day of disease	Inoculum	Route	Result
64	An afebrile patient	27-28	Lice excreta	Scarified skin	+
70	" "	"	" "	" "	+
73	" "	"	" "	" "	+
75	" "	"	" "	" "	+
77	" "	"	" "	Conjunctiva	+
81	" "	"	" "	Scarified skin	+
84	" "	"	" "	" "	+

These experiments were made by feeding lice once on a Trench Fever case during an afebrile period, and afterwards using their excreta to infect healthy men. All gave a positive result, but fail in their object, since the patient again became febrile on the afternoon of the day on which the lice were fed.

8. *To ascertain at what temperature lice require to be kept between feedings, in order that they may become infected.*

Table XVIII.

Exp. no.	Amount of excreta	Temperature lice kept at	Route	Result
146	From 200 lice	30° C.	Scarified skin	-
147	" "	12°-17° C.	" "	-
117	1 mgm.	27° C.	" "	+
118	1 mgm.	17° C.	" "	+

These experiments are merely placed on record. From Exp. 118 it is evident that lice kept at room temperature between feedings are capable of

carrying infection. The experiments are few in number and irregular in result but indicate that a temperature between 17° C. and 27° C. is adequate.

9. *To ascertain what time elapses between an infecting feed and the infectivity of the louse excreta.*

Table XIX.

Exp. no.	Interval in days between infection of lice and collection of excreta	No. of lice used	Route	Result
23	1	500	Scarified skin	-
39	1	...	" "	-
24	3	400	" "	-
25	5	400	" "	-
53	5	...	" "	+
41	6	...	" "	-
54	6	...	" "	-
42	7	...	" "	+
55	7	...	" "	+
26	8	400	" "	-
43	8	...	" "	-
91	8	...	" "	-
45	9	...	" "	+
46	10	...	" "	+
59	11	...	" "	+
27	12	...	" "	+
30	23	...	" "	+

It is shown by the above table that the excreta were first found to be infective five days after the infected feed. From the ninth day the results were invariably positive. It would appear from this that the organism of Trench Fever undergoes multiplication in the intestine of the louse and that from the fifth day onwards they may be so numerous in the excreta as to be capable of infecting healthy men.

10. *To ascertain what percentage of lice fed upon trench fever cases become infected.*

Table XX.

Exp. no.	Inoculation of	Route	Result
60	Excreta of 10 lice	Scarified skin	+
66	" " 5 "	"	+
71	" " 1 louse	"	+
79	" " 1 "	"	+

Nature of experiment. Ten clean lice were fed twice daily on a Trench Fever case for a period of 18 days. The excreta passed during the last three days of that time, that is the 16, 17 and 18th days, were collected and inoculated into a scarified skin area (Exp. 60).

Meanwhile the lice had been separated into two batches of five each, and fed as usual on the same patient. The excreta passed by one batch of five were collected during the next seven days and inoculated into the skin (Exp. 66).

Two lice only of this same batch of five continued to live and thrive. For some time past the individual lice had been housed separately in glass tubes and kept in an incubator at 32° C.

The excreta of one of these lice were collected for five days and inoculated into a healthy man (Exp. 71); from the other in the same way into another volunteer (Exp. 79). All these experiments proved to be positive.

It may therefore be concluded that a high percentage of lice become eventually infected; and that the excreta from one louse collected for five days may be sufficient to produce a severe and typical attack of Trench Fever.

11. *Hereditary transmission of the organism in the louse.*

Table XXI.

Exp. no.	Nature of experiment	Parents fed on	Stage of offspring	Route	Result
22	Inoculation of excreta of offspring of infected lice	Various cases	Adult	Scarified skin	-
121	Inoculation of excreta of offspring of infected lice	One case	"	"	-
129	Inoculation of excreta of offspring of infected lice	"	"	"	-

From these three experiments it would appear that the microorganism of Trench Fever is not passed from infected lice to their offspring.

It may be added that the excreta of the parent lice had been proved to be infective. Great care was taken to prevent the carriage of infective material on the outside of the eggs. They were washed thoroughly in lysol and afterwards in sterile water, dried and placed in clean boxes. When hatched out they were fed on a healthy person until they were adults. After the young lice had fed as adults for the necessary period (10 to 15 days or more) and having been kept in the proper temperature their excreta was collected and used in the above experiments.

12. *To ascertain if Pediculus capitis can convey the virus of trench fever.*

Only one experiment (No. 151) was made. The head-lice were fed in the usual way on a Trench Fever case; their excreta collected and rubbed into a scarified area of the skin of a healthy volunteer. The result was positive. We may therefore conclude that *P. capitis* can also carry the organism of Trench Fever from the sick to the healthy.

13. *To ascertain the minimal infecting dose of louse excreta.*

Table XXII.

Exp. no.	Amount of excreta	Treatment of excreta		Route	Result
102	0.05 mgm.	Dissolved in 1 c.c. N.S.		Sub-cutaneous	-
109	0.1	"	"	"	-
97	0.1	"	"	"	+
117	1.0	"	"	"	+
98	2.0	"	"	"	+

From the above five experiments it would appear that a tenth of a milligram of excreta is sufficient to give rise to the disease.

VI. VIABILITY OF THE TRENCH FEVER ORGANISM.

1. *To ascertain the effect of drying on the organism contained in the blood of trench fever cases.*

Table XXIII.

Exp. no.	Amount injected	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
137	5 c.c.	1	Dried over sulphuric acid for 39 hours	42 hours	Subcutaneous	-
175	10 c.c.	5	Dried over sulphuric acid	46 hours	Intramuscular	-

Two experiments, Nos. 137 and 175, were made on the effect of drying Trench Fever blood. The blood was dried over sulphuric acid for about 40 hours; the residue redissolved and injected into two healthy men.

Both experiments were unsatisfactory as the controls also failed to take the disease. The experiments were made on the analogy of South African Horse-sickness, the ultramicroscopical organism of which is said to be destroyed by drying. It seems very unlikely in the case of Trench Fever that the organism will be so easily destroyed in the blood seeing that the dried excreta of infected lice retain their power of infection for a long time. But it is possible that the organism in the blood and in the excreta may differ in their powers of resistance.

Conclusion. The effect of drying on the virus of Trench Fever contained in the blood remains unproven; if an opportunity of repeating the experiment is found it should be taken.

2. *Exposure to sunlight.*

One experiment (No. 21) was made to test the effect of sunlight on the Trench Fever organism. The dried excreta of infected lice were placed in a glass tube and exposed frequently to sunlight for 16 days. At the end of this time the excreta were rubbed into a scarified area of skin of a healthy man. The result was positive. It may therefore be concluded that exposure to sunlight for several days does not sterilise infected excreta.

No attempt was made to try the effect of sunlight on the virus in the blood.

3. *Effect of distilled water on the virus.*

Table XXIV.

Exp. no.	Amount of excreta	Amount of distilled water	Time of exposure	Route	Result
63	Rest on sixpence	1 c.c.	24 hours	Skin	-
150	4 mgm.	1 c.c.	10 minutes	Subcutaneous	+
164	4 "	1.5 c.c.	10 "	"	+
165	4 "	1.5 c.c.	30 "	"	+

These experiments arose out of Exp. 5 in which 2·7 c.c. of infected blood, 10 c.c. of which had been laked by the addition of 170 c.c. distilled water, failed to give rise to the disease. Exp. 63 was also negative but was not satisfactory on account of the technique used, and also because the man was suspected of being naturally immune. The other three experiments were positive and prove that distilled water has no effect on infective excreta up to an exposure of 30 minutes.

Conclusion. There is some evidence, although unsatisfactory, that distilled water destroys the virulence of the virus in blood, and also in excreta if the exposure is prolonged. Exposure up to 30 minutes is without effect.

4. *How long do louse excreta remain infective?*

Table XXV.

Exp. no.	Amount of excreta	Temp. kept at	Length of time	Nature of experiment	Route	Result
57	...	Room	60 days	Rubbed in	Skin	+
95	...	"	120 "	"	"	+
178	8 mg.	"	275 "	In 1·5 c.c. N.S.	Subcutaneous	-
195	40 "	"	365 "	"	"	-

The excreta were stored in glass tubes plugged with cotton-wool and kept at the temperature of the laboratory. Exp. 178 in which the excreta had been kept for nine months, seems satisfactory, but Exp. 195 not so, as the man when tested afterwards for immunity failed to react.

Conclusion. It would appear that the organism in the excreta of infected lice retains its virulence for at least four months when kept in plugged glass tubes at room temperature.

5. *At what temperature is the organism in lice excreta killed?*

(a) Dry Heat.

Table XXVI.

Exp. no.	Amount of excreta	Temperature	Time	Nature of experiment	Route	Result
33	Not stated	56° C.	20 minutes	Rubbed in	Skin	+
58	" "	65° "	20 "	"	"	+
73	" "	70° "	20 "	"	"	+
84	" "	80° "	20 "	"	"	+
94	" "	100° "	20 "	"	"	-

It would appear from the above experiments that lice excreta retain their virulence after exposure to a dry heat of 80° C. for 20 minutes; but lose it at a temperature of 100° C.

(b) Moist Heat.

Table XXVII.

Exp. no.	Amount of excreta	Temperature	Time	Nature of experiment	Route	Result
34	Not stated	80° C.	10 minutes	Rubbed in	Skin	-
82	" "	70° "	20 "	"	"	-
83	" "	60° "	20 "	"	"	-

From these three experiments it would appear that the organism of Trench Fever is destroyed when exposed to a moist heat of 60° C. for 20 minutes.

The method of carrying out these experiments on the action of dry and moist heat is detailed by Byam and his colleagues in their book entitled *Trench Fever*, in the chapter on prophylaxis. They state that the American Red Cross Commission on Trench Fever found that the virulence of the excreta was not destroyed by exposure to 60° C., moist heat, for 30 minutes, but that it was destroyed by exposure to 70° C., moist heat, for a similar time. They are unable to account for this discrepancy.

It is unnecessary to go further into the question of the destruction of lice, their eggs and the virus of the excreta in this report, since all these points are dealt with in the above-mentioned book and in various papers the titles of which are given in the bibliography.

6. *Effect of ultra-violet rays on the organism.*

Table XXVIII.

Exp. no.	Amount of excreta	Dose of rays	Time	Route	Result
62	Not stated	Until all the bacteria were killed	Not stated	Skin	+
155	" "	4 times lethal dose for anthrax spores	" "	"	+
134	4 mgm.	3 times lethal dose for bacteria	" "	Subcut.	+
168	4 mgm. in 1.6 c.c. N.S.	Fully lethal 4 times for most organisms	30 minutes	"	+

These experiments were carried out by Mr Barnard, one of the members of the Committee. The lice excreta were exposed in Exp. 155 to four times the lethal dose for anthrax spores. They gave bacterial growth before but none after exposure, in either aerobic or anaerobic cultures. For details the different experiments should be consulted. Barnard states in regard to Exp. 134 that the excreta received three times the lethal dose of ultra-violet light for ordinary bacteria. If however the organisms were smaller than the mean wave-length he was using they would escape destruction.

7. *Effect of antiseptic and other chemical substances.*

Table XXIX.

Exp. no.	Amount of excreta	Chemical	Time	Temperature	Route	Result
74	Not stated	2 % lysol	20 minutes	20° C.	Skin	-
75	" "	2 % Army soap and tap-water	20 "	47.6° to 38° C.	"	+
80	" "	2 % cresol	22 "	18.5° C.	"	-
209	0.2 gm.	3 c.c. glycerine and 3 c.c. N.S.	10 days	...	"	-
211	3 mgm.	Equal parts of glycerine and N.S.	7 days	...	Subcut.	+

These experiments go to show that 2 per cent. of lysol or cresol solution is sufficient to render lice excreta harmless, but an exposure of seven days to equal parts of glycerine and salt solution has no effect.

VII. ARE ANY OF THE LABORATORY ANIMALS SUSCEPTIBLE TO TRENCH FEVER?

This question must be answered for the present in the negative, or at least as not proven. Arkwright inoculated several monkeys with infective excreta, but none of the animals showed any marked reaction. Lice were fed on one of them, and their excreta afterwards used to inoculate Exp. 61 who however did not react in the slightest degree.

Ledingham on his return from Active Service in May 1919 took up the question of the transmissibility of the Trench Fever virus to laboratory animals. He summarises the results as follows:

(1) Experiments (about 50 in all) undertaken with the object of transmitting Trench Fever to laboratory animals proved in the main negative. A very small minority of inoculated rabbits and guinea-pigs, however, exhibited temperature charts which at least suggested a successful transmission of the virus. The question must for the present remain undecided, but it is probable that a certain small percentage of normal rabbits and guinea-pigs may be susceptible to the virus.

(2) In the course of the work it was established that the *Rickettsia* present in the excreta of lice fed on Trench Fever patients are agglutinated in the presence of immune serum obtained from animals immunised with infective lice excreta.

(3) Trench Fever *Rickettsia* are not agglutinated by the serum of animals immunised with the excreta of normal uninfected lice.

Ledingham failed to obtain samples of blood from recent cases of Trench Fever in order to ascertain whether the reaction possesses a diagnostic value. None of the old chronic cases which were available gave any reaction.

VIII. IMMUNITY.

1. *To ascertain what percentage, if any, of men are naturally immune to trench fever.*

The subjects of the eleven experiments given in Table XXX were men who had been subjected to various experiments to which they had not reacted. For example No. 145 had passed through four such experiments, the last of which consisted of the inoculation of 2 mgm. of infective excreta. To ascertain if he were really immune 8 mgm. were given, but to this large dose he also failed to react. It is possible that the previous four experiments had in some way brought about this immunity, but if not, then this man must be regarded as a case of natural immunity. In the same way Nos. 160 and 197 may be looked on as cases of natural immunity. Out of all the men used for these experiments, some 216 in number, only three were detected as being probably naturally immune.

It may then be concluded that only a very small proportion of the population are naturally immune to the disease.

Table XXX.

Exp. no.	Previous negative experiments	Amount of excreta	Treatment of inoculum	Route	Result
	No.				
145	{ 94. Heated excreta	8 mgm.	0.5 c.c. N.S.	Subcutaneous	-
	{ 103. 0.05 "				
	{ 109. 0.1 "				
	{ 130. 2 mgm. "				
160	{ 140. Distilled water	"	"	"	-
	{ 154. Lice kept at 30° C.				
162	{ 131. Lice excreta	"	"	"	+
	{ 144. Lice fed once				
	{ 156. One louse				
163	{ 88. Minimum quantity of blood	"	"	"	+
	{ 100. One louse				
	{ 107. One louse				
179	{ 153. Filtration	"	"	"	+
	{ 159. Urine				
186	{ 149. Feeding lice	Not stated	None	Skin	+
	{ 178. Lice 275 days old				
189	174. Whole blood	"	1.5 c.c. N.S.	Subcutaneous	+
196	{ 188. 1st day of disease	"	None	Skin	+
	{ 191. " "				
	{ 194. Excreta of lice				
197	{ 173. 0.2 c.c. blood	"	None	"	-
	{ 195. Excreta 1 year old				
201	{ 201. Small dose excreta	5 mgm.	N.S.	Subcutaneous	+
	{ 201. " "				
	{ 201. " "				
210	206. Supposed case	Not stated	0.7 c.c. N.S.	"	+

2. *To ascertain if one attack of trench fever confers immunity.*

This is an important question and one which it is difficult to answer categorically. It must be remembered that the disease is often an extremely chronic one. Lice fed on two patients 298 and 443 days after the onset of the fever (Exps. 72 and 181) became infected, showing that the virus still persisted in the patient's blood. The fact of the Trench Fever parasite being able to live so long in the blood is an argument that the tissues of the body have little power of dealing with it. It would be well in testing for immunity to make sure that the patient was completely recovered from his first attack. If a case has still the living virus in his blood it is not to be wondered at if he shows no reaction to the testing dose. In the same way it is not to be wondered at if the case does show a reaction, the slight addition of poison being enough to upset the equilibrium.

Byam and his fellow workers made many experiments to test this question, but in few of them was it first proved that the case was free from infection or had completely recovered. Many of them were obviously still suffering from the disease. The experiments are divided into different categories.

In the first place let us consider the effect of the disease contracted naturally in France. The men were admitted to the Hampstead Hospital suffering from Trench Fever or its sequelæ. After they had apparently recovered, the tempera-

ture being normal and the signs of acute disease having disappeared; they were tested as to their immunity to further attack by being inoculated with Trench Fever blood or infected lice excreta.

The following table gives the result of the attempt to reinfect with Trench Fever blood injected intravenously. The average number of days since the onset of disease is 85 days.

(a) *To ascertain if men who have suffered from trench fever acquired naturally, are immune to a second attack when trench fever blood is injected intravenously.*

Table XXXI.

Exp. no.	Time after onset of disease	Amount of blood	Route	Condition when tested	Result
35	62 days	5 c.c.	Vein	Pain in limbs	-
37	99 "	5 "	"	D.A.H. and debility	-
49	98 "	5 "	"	Pain in legs	-
65	119 "	4 "	"	Fit	-
108	80 "	20 "	"	Pains in head and legs	-
110	85 "	20 "	"	Pains in head and fever	-
111	70 "	20 "	"	Pains in head, spleen enlarged	+
112	65 "	20 "	"	D.A.H. and pains in limbs	+

Table XXXI shows that of eight cases, six gave a negative reaction, and two a positive. With the exception of one, all were still suffering from the effects of the fever. The number of positive reactions is small, which may be accounted for by the fact that Trench Fever blood at no time seems to contain much of the virus.

It is evident that these cases do not answer the question as to whether one attack confers immunity or not.

(b) *To ascertain if men who have suffered from naturally acquired trench fever are immune to a second attack when infected lice excreta are inoculated.*

Let us see what is the effect of the inoculation of infected lice excreta on these apparently recovered cases. As lice excreta are more virulent than Trench Fever blood, more positive results would be anticipated.

Table XXXII gives a list of the men who acquired the disease naturally and were afterwards tested by lice excreta, either rubbed into a scarified area of the skin or made into an emulsion and injected subcutaneously. The average number of days since the onset of the disease is 114 days.

Table XXXII.

Exp. no.	Time after onset of disease	Amount of excreta	Condition when tested	Route	Result
20	120 days	Not given	Bradycardia	Skin	+
51	115 "	"	D.A.H. debility	"	+
52	117 "	"	Dyspepsia and pains	"	+
76	134 "	"	Giddiness and pains	"	-
85	48 "	"	Headache and pains	"	-
86	52 "	"	Pain in back and legs	"	+
90	149 "	"	Slight fever and dizziness	"	-
104	194 "	8 mgm.	Fit	Subcutaneous	+

Here again there are eight cases, but now five are positive and only three negative. In one of the negative cases the infectivity of the louse excreta was doubtful and in the other two, the lice were fed on the patients themselves. If these three unsatisfactory cases are left out, then all the remainder are positive, with an average incubation period of 10 days.

It may therefore be asserted that cases of Trench Fever with an average of 114 days from the onset of the disease will as a rule show a definite reaction if inoculated with infected lice excreta. Whether this is really a fresh attack of the disease or merely a relapse or superinfection is doubtful. It may be noted that only one of the men is reported as being fit, all the others were still suffering from the effects of the fever. It will also be remembered that in some cases lice fed on old cases up to 443 days after the onset of the disease became infected, showing that the organism was still present in the blood. These experiments would go to show that the production of immunity in man is slow and imperfect.

(c) *To ascertain if men who have passed through one attack of trench fever, artificially acquired, are immune to a second attack, when trench fever blood is injected or lice excreta inoculated.*

It now remains to enquire into the cases which were given the disease artificially by the inoculation of Trench Fever blood or infected lice excreta. These were the volunteers who had been successfully infected with the fever in the course of various experiments. Table XXXIII gives the experimental numbers of these cases, their condition as to health when tested, the length of time which had elapsed since the onset of the fever, the method of testing, whether by blood or lice excreta, and the result.

Table XXXIII.

Exp. no.	Time after onset of disease	Condition when tested	Amount of blood or excreta	Route	Result
28	30 days	Pains	Uncertain	Skin	-
29	31 "	Palpable spleen	"	"	-
70	139 "	Fit	"	"	+
89	119 "	"	20 c.c.	Vein	-
98	154 "	"	Uncertain	Skin	-
99	132 "	"	"	"	+
103	48 "	"	20 c.c.	Vein	-
105	182 "	"	20 mgm.	Subcutaneous	-
114	76 "	"	4 mgm.	"	-
115	122 "	"	50 c.c.	Vein	-
116	116 "	"	"	"	-
125	139 "	"	"	"	-

There are twelve of these cases with an average of 108 days since the onset of the disease. Of these two were still unfit, the remainder fit. Only two out of the twelve gave a positive reaction, the other eight proved refractory to the testing dose. One, No. 105, was tested with large doses on three separate occasions, the last time six months after the onset of the fever.

Conclusion. With the data at our disposal it is not possible to give a categorical answer to the question, does one attack of Trench Fever confer immunity? Taking everything into consideration, it is probable that one attack does not confer immunity as, for example, is understood in measles, scarlet fever or small-pox; but rather a partial and limited immunity as in influenza or pneumonia.

IX. ETIOLOGY.

1. *Rickettsia-like organisms found in the excreta of infected lice.*

Three members of the War Office Trench Fever Committee, the late Professor Plimmer, F.R.S., and Dr Arkwright and Mr Bacot of the Lister Institute, devoted themselves to the search for a causal organism in Trench Fever. Professor Plimmer exhibited preparations made from the excreta of infected lice which showed enormous numbers of very small bodies. These bodies resembled the *Rickettsia* described by Töpfer and Rocha-Lima in 1916 as occurring in lice of Trench Fever patients, and led Arkwright and Bacot to follow up this line of research. It is not necessary here to consider their results in detail as they have been fully described in a paper entitled "The Association of *Rickettsia* with Trench Fever," by J. A. Arkwright, A. Bacot and Martin Duncan (*Journ. Hygiene*, xviii. 76-94, Pls. II, III). It will be sufficient here to quote their conclusions:

Conclusions.

1 The intimate association in lice of *Rickettsia* with the virus of Trench Fever appears to have been amply proved.

2. The examinations of lice which have fed on healthy civilians in England have given negative results in a sufficiently uniform manner to constitute a significant negative control, but further examinations of lice from normal civilians are desirable.

3. Lice from soldiers who have been in France, or who have mixed with men from France in this country, would not afford a satisfactory control, since the infection of Trench Fever with the power of infecting lice with Trench Fever virus and *Rickettsia* may be very long lasting.

4. Whether *Rickettsia* constitutes the virus of Trench Fever or are in some way produced by it remains undecided because *Rickettsia* cannot be cultivated on artificial media.

5. It is conceivable that *Rickettsia* are not living microorganisms, but their appearance certainly suggests that they are bacteria, and their remarkable association with Trench Fever virus in the louse further suggests that they are the causal agent of Trench Fever.

2. *To ascertain if lice which show Rickettsia in their excreta are infective.*

The following experiments on the relation of the presence of *Rickettsia* in louse excreta to the infectivity of the excreta were made by Major Byam and his co-workers at Hampstead Hospital on behalf of Arkwright and his colleagues and are simply recorded here as they are fully discussed by these workers in their paper quoted above.

Trench Fever

Table XXXIV.

Exp. no.	Nature of experiment	<i>Rickettsia</i>	Treatment of inoculum	Route	Result
100	Gut contents 1 louse	—	Rubbed into scarified area	Skin	—
101	"	+	"	"	+
142	1 louse	—	"	"	—
143	"	+	"	"	+
156	"	+ +	"	"	—
157	"	—	"	"	+
166	"	—	"	"	—
167	"	+	"	"	+
106	"	—	N.S.	Subcutaneous	—
107	"	+	"	"	—
122	"	—	Rubbed in	Skin	—
123	"	+	"	"	—
144	Excreta	—	"	"	—
148	"	+	"	"	+

3. *To ascertain how early in the disease a patient can cause lice fed on him to show Rickettsia.*

A number of boxes containing healthy lice were prepared. Each box was fed for one day on a case of Trench Fever. The box was then fed on a healthy man and the excreta examined for *Rickettsia* from day to day. Table XXXV gives the result.

Table XXXV.

No. of box of lice	Fed on case of Trench Fever	<i>Rickettsia</i> showed	Result
268	1 day before onset, 3 feeds	12th day	+
269	1st day	—	—
276	1st "	—	—
271	2nd "	9th day	+
272	3rd "	—	—
273	4th "	8th day	+
274	5th "	9th "	+
275	6th "	8th "	+
276	7th "	7th "	+
277	8th "	10th "	+

It is evident from these experiments that lice fed on Trench Fever cases in the first days of the illness, in one case on the day preceding the onset of the fever, developed *Rickettsia* in the excreta. The experiments also show on what day after the infecting feed these bodies appear. The earliest is the seventh day, the latest the twelfth, the average the ninth.

X. TO ASCERTAIN IF THE TRENCH FEVER ORGANISM CAN BE TRANSMITTED BY OTHER BLOOD-SUCKING INSECTS.

1. *By the bug.*

An experiment (No. 113) was made in order to find out if Trench Fever can be transmitted by the bites of bugs. The box of bugs was fed on a Trench

Fever case for nearly a month, their excreta collected and injected into a healthy man. The result was negative.

Another experiment (No. 158) was made to ascertain if *Rickettsia* appeared in the excreta of the bug after feeding on a Trench Fever case. The bugs were only fed once on the first day of the disease. It was thought that *Rickettsia* did show in the excreta, but these proved negative when inoculated into Exp. 161. Exp. 158 is unsatisfactory as lice fed at the same time and on the same patient did not develop *Rickettsia* nor were they infective. In connection with this it may be pointed out that a hereditary *Rickettsia*-like parasite of the bed bug has been described by Arkwright, Atkin and Bacot in *Parasitology*, vol. XIII. No. 1, 14th March, 1921.

From this one experiment it may be concluded that probably the bug does not serve as a carrier, but more experiments are required to settle the question.

XI. TO ASCERTAIN IF THE ORGANISM OF TRENCH FEVER CAN BE CULTIVATED IN ARTIFICIAL MEDIA.

Three experiments (192, 193 and 206) were made by injecting supposed sub-cultures of the Trench Fever organism grown on Noguchi's medium into three volunteers. These were all negative.

Up to the present then there is no proof that the organism of Trench Fever can be grown on any artificial culture medium.

XII. FILTERABILITY OF THE TRENCH FEVER ORGANISM.

1. *When present in the blood.*

Table XXXVI.

Exp. no.	Amount injected	Day of disease	Treatment of blood	Interval between collection and experiment	Route	Result
6	2.3 c.c.	9th	10 c.c. blood laked in 170 c.c. Aq. dist. added to saline	1 hour 30 mins.	Vein	-
206	12 c.c.	6th	10 c.c. blood added to 4 c.c. Sod. Cit. Sol. centrifuged for 1 hr. 54 mins. decanted Barnard's filter	3 hours 43 mins.	„	-
216	5 c.c.	4th	60 c.c. blood citrated, settled by gravity, 10 c.c. plasma, Barnard's filter	9½ hours	„	-

There were only three filtration experiments made by the War Office Committee with the blood of Trench Fever cases. The first, Exp. 6, was made with laked blood, but as the same laked blood without filtration also gave a negative result, Exp. 6 may be considered a wash-out. The blood which was used in this experiment had been proved to be infective, and its loss of infectivity was supposed to be due to the distilled water used in the process of laking.

The other two experiments were made by Mr Barnard to test collodion

filters. One was positive and one negative. It is to be hoped Mr Barnard will pursue this line of investigation and publish the results. In the mean time nothing can be concluded from these two experiments.

McNee, Brunt and Renshaw made several experiments with blood in order to establish whether or not the Trench Fever virus is a filter-passer. They do not commit themselves to any definite opinion, but as their hypothesis was that the Trench Fever microorganism is an intra-corpuscular one they were driven to the position that neither the plasma nor the serum could be infective unless broken down corpuscles were contained in it.

The American Red Cross Commission also attempted to filter the virus in the blood, but with negative results. They considered that this was due to the clogging of the pores of the filter by the blood, as from other experiments they had come to the conclusion that the virus is ultra-microscopical and a filter-passer.

2. When present in the excreta of infected lice.

The American Red Cross Commission, having failed to filter the virus in blood plasma or serum, turned their attention to the virus in the urine of patients and in the excreta of lice. Five experiments were made; three of these were positive, two negative.

Two experiments with urinary sediment filtered through an unglazed Chamberland filter gave one positive and one negative result. Three experiments with filtered louse excreta gave two positive and one negative result. The filters were tested before and after the operation with *Bacillus typhosus*.

From these experiments they conclude that at least one stage of the development of the virus of Trench Fever is filterable and ultra-microscopical.

The War Office Committee made eight experiments with filtered emulsions of lice excreta, a summary of which is given in Table XXXVII. Two of these were positive and six negative. Four were made through Chamberland F filters, four through Berkefeld filters.

Table XXXVII.

Exp. no.	Amount injected	Treatment of excreta	Route	Result	Remarks
153	20 c.c.	1 % suspension of louse excreta	Vein	-	Chamberland F., pressure 350 to 400 mm. Hg.
169	50 "	1 in 400 N.S.	"	-	Chamberland filter, pressure 100 mm. Hg.
170	50 "	" "	"	-	Chamberland filter, pressure 100 mm. Hg.
171	50 "	" "	Subcutaneous	-	Chamberland filter, pressure 100 mm. Hg.
187	10 c.c.	0.1 gm. emulsified in N.S.	"	+	Berkefeld filter, pressure 200 to 300 mm.
190	10 "	1 % suspension of louse excreta	Vein	-	Berkefeld filter, pressure 300 to 350 mm.
199	35 "	0.5 gm. excreta in 100 c.c. N.S.	Subcutaneous	+	Berkefeld filter D., pressure 600 to 740 mm.
200	49 "	0.5 gm. excreta in 100 c.c. N.S.	"	-	Berkefeld filter, E., pressure 600 to 740 mm.

Conclusion. The virus of Trench Fever cannot be said to be ultra-microscopical and a filter-passer in the sense these terms are used at the present day. It may rather be concluded that the organism is small since it succeeds in passing through a Berkefeld filter on two occasions. Such a body about the size of the so-called *Rickettsia* would seem to fulfil the conditions, and the result of the filtration experiments would appear to be rather more in favour of the hypothesis that *Rickettsia* are the cause of the disease than against it.

SUMMARY.

1. The organism of Trench Fever is present in the "whole blood."
2. The evidence seems to be in favour of the organism being an extra-corpuscular rather than an intra-corpuscular parasite.
3. The smallest quantity of whole blood which has given rise to the disease was 0.5 c.c.
4. The blood is infective from the first day of the disease, and in one case was found to be still infective after 443 days.
5. The microscopical examination of the blood has not up to the present revealed the organism or at least has not differentiated it from other granules present in blood.
6. There is some evidence that the organism may leave the body in the sputum and urine, but the chief and only way which has any practical significance is by means of a blood-sucking insect, the louse.
7. There is no evidence that infection takes place through food, drink or air, but only by inoculation of the organism by means of this insect.
8. The chief method of infection is not, as might be expected, by the bite of the louse, but by the infected excreta of the louse being brought in contact with an abraded surface of the skin.
9. After a louse has fed on a case of Trench Fever, five to nine days elapse before the excreta become infective.
10. If lice are fed on a case for some time a high percentage of the lice become infective.
11. There is no transmission of the organism of Trench Fever from infected lice through the egg to their offspring.
12. The tenth of a milligram of excreta has been found sufficient to set up the disease.
13. The organism of Trench Fever retains its virulence in louse excreta for at least four months.
14. The virulence of lice excreta is lost by exposure to dry heat of 100° C. for 20 minutes; to moist heat of 60° C. for a similar time.
15. It is doubtful if any of the laboratory animals are susceptible to Trench Fever.

16. It would appear that most men are susceptible to the disease, only a very few individuals having been found apparently naturally immune.

17. One attack seems to produce only a partial and limited immunity.

18. In regard to the etiology of Trench Fever there is strong evidence that the *Rickettsia*-like bodies found in infected louse excreta are the micro-organisms of the disease, but as they have never been cultivated outside the body the final proof is still wanting.

19. The organism cannot be said to be a filter-passer; the evidence would seem to be more in favour of its being a small body such as the so-called *Rickettsia*.

SOME POINTS IN THE EPIDEMIOLOGY OF AN OUTBREAK OF CEREBRO-SPINAL FEVER IN HONG KONG, 1918.

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(With 3 Charts, 2 Maps and 1 Figure.)

TABLE OF CONTENTS.

	PAGE
I. Pre-epidemic Period	289
Outbreak in Manila	289
Evidence as to existence of sporadic cases of cerebro-spinal fever in Hong Kong	290
Pre-epidemic infectious diseases	290
II. Diagnosis—Onset and Course of Epidemic	291
Daily incidence of cases (with Chart No. 3)	291
Mortality	291
Missed cases	292
Age and sex incidence (with Charts Nos. 1 and 2)	293
Incidence among Europeans	295
III. Meteorological Conditions (with Chart No. 3)	297
Conclusions as to influence on the course of the epidemic	299
IV. Geographical Distribution and Overcrowding	299
Distribution of the population	299
Estimation of population of the city of Victoria	301
Density per acre	301
Migration of the population	302
Housing conditions in Hong Kong	303
Spot maps (Maps I and II)	306
V. Habits of the Chinese	308
VI. Influence of Length of Residence in the Colony on Case Incidence	309
VII. Occupation Incidence	310
VIII. Carrier Relation to the Epidemic	311
IX. Summary of Conclusions	316

I. PRE-EPIDEMIC PERIOD.

PREVIOUS to 9th February, 1918, the date on which it was first recognised that an epidemic of cerebro-spinal fever, or epidemic meningitis prevailed in Hong Kong, there existed no records of any former epidemic in Hong Kong, or on the China coast. In 1915, however, an epidemic of 70 known cases occurred in the Philippines, of which Manila is the next port of call to Hong Kong of the trans-Pacific steamships.

In November, 1917, a few weeks after taking up office as Medical Officer of Health, on compiling mortality statistics, I observed that in the previous six months, from May until October, 67 certificates of death from "meningitis" undefined were issued in the colony. Of these 67 cases, all of Chinese nationality, 46 were issued from one hospital out-patient department, and 38 of the death certificates were signed by one medical man, Dr X. I asked Dr X if he had any reason to suspect any of these cases to be sporadic cerebro-spinal fever, and he replied that he could not say, but that these Chinese cases were mostly children brought to him at the point of death with obvious signs of meningitis. He agreed to enquire more particularly into future cases and to take, when possible, specimens of cerebro-spinal fluid and submit them to the bacteriological department for diagnosis. One such sample was submitted for diagnosis without result.

In the mortality statistics for the year 1917, compiled by myself, there were reported 57 deaths from "tubercular meningitis," including one mortuary return, and 99 deaths from "meningitis" undefined, including three mortuary returns, a total of 156 deaths from some form of meningitis. In the sanitary reports of the years previous to this the number of deaths from tubercular meningitis was not stated, but for 1916 the number of deaths under the heading of nervous diseases labelled "meningitis" was 142, of which one only was a mortuary return: for 1915 the similar return was 17, including one mortuary case: for 1914 there were 93 cases, including three mortuary returns further differentiated as septic meningitis. These meningitis returns from 1914-1916 inclusive must be taken to be in addition to the returns of deaths from tubercular meningitis which, in the Hong Kong reports, were included under the heading of tubercular disease.

If posterior basic meningitis in young children¹ is accepted as identical with sporadic cases of cerebro-spinal fever, it is probable that these returns (varying from 93, 17, 142 to 99 in four successive years), with the further observation that three cases in 1914 were reported from the mortuary as septic meningitis, point to the conclusion that cerebro-spinal fever existed to some extent in the colony before 1918.

Pre-epidemic infectious disease. It was the subject of much comment during the months of December, 1917, and January, 1918, that a very widespread infection of influenza colds, or catarrhal pharyngitis, of varying severity existed in the colony. The weather during this period was ideal, clear, cold and constant brilliant sunshine, but owing to the lack of rain there was a good deal of dust flying about and it was the fashion to ascribe these attacks to dust irritation, or infection. At this time measles also existed in the colony, but to a quite unknown extent, as measles is not a notifiable disease in Hong Kong. From January 28th to February 5th eight cases of death from haemorrhagic smallpox were reported from the mortuary, and on February 5th and February 6th four additional cases were reported. As smallpox was

¹ *Cerebro-spinal Fever in Camps or Barracks*, by Sir W. Osler, M.D., F.R.S., 30. 1. 15.

non-existent in the colony at this time, the occurrence of 12 consecutive cases of haemorrhagic smallpox and no single case of ordinary smallpox gave rise to discussion and the suspicion that the diagnosis might be mistaken.

From the date of the recognition of the epidemic on February 9th no further cases of haemorrhagic, or ordinary, smallpox were reported for some months, and, as those responsible for the notification of these cases agreed that they were identical in appearance with many of the cases of cerebro-spinal fever brought in dead, it may be assumed that these were also cases of cerebro-spinal fever and that the epidemic had already existed in the colony for some time before it was recognised.

During the last two weeks in January it was reported from the Tung Wah hospital for Chinese that several cases had died in an acute typhoid condition, that the Widal reaction had been consistently negative, but that the death certificate was signed typhoid fever in lieu of a better diagnosis. In view of subsequent experience of the epidemic in which many of the verified cases presented an identical clinical picture with these other suspicious typhoid cases, it was considered that the former diagnosis of typhoid fever should be changed to that of cerebro-spinal fever.

II. DIAGNOSIS—ONSET AND COURSE OF EPIDEMIC.

On February 9th a telephone message was received that there were several cases in hospital suspected to be suffering from cerebro-spinal fever. Most of these cases had been for a few days in hospital but it was not until a typical case with meningeal symptoms was seen and the cerebro-spinal fluid drawn off that suspicion was cast on the others.

On February 11th eight verified cases were reported and from that date the epidemic steadily progressed.

The epidemic reached its height in March with an average of 14 cases reported per day, the highest number recorded for any one day being 24, on March 26th, out of which 18 were brought in dead or died the same day as discovered (see Chart No. 3, p. 296).

For the purposes of this paper the records from February 9th to June 1st are taken, at which date the epidemic was gradually tailing off, and includes a total of 1040 cases. The doubtful typhoid and haemorrhagic small pox cases are not included.

The following table is arranged to show for each month the average number of cases per day, the average number of cases brought in dead per day, the highest number recorded in any one day of new cases, and cases found dead or dying.

Table A.

	Feb.	March	April	May
Average No. cases per day	9	14	9	4
" " " brought in dead ...	4.4	7	4.5	2.6
Highest No. cases recorded for one day ...	18	24	14	10
" " brought in dead for one day	6	18	9	6

Out of the 1040 known cases, 889 died, which gives a mortality of 85.48 per cent., but this includes those cases, 519 in all, brought in dead or *in extremis*.

It is interesting to note that the curve of the number of cases daily brought in dead, or *in extremis*, follows the curve of the daily incidence of cases (see Chart No. 3, p. 296).

This is of special interest when viewed in the light of Magelssen's observations in Copenhagen. He demonstrated by curves "that fluctuations in mortality cannot be ascribed to the merit of hygiene alone. They seem to be dependent on the temperature or some unknown factor closely associated with it, which controls the fluctuations in the mortality, the *constitutio epidemica*."

In Hong Kong, where the epidemic was practically uncontrolled, this fluctuation is very well marked and bears a definite relationship to the meteorological conditions, as is shown in Chart No. 3 (p. 296), discussed under the heading of meteorological conditions.

Missed cases. There is no doubt that this number, 1040, does not by any means represent the total number of cases which occurred. To this must be added:

1. Those cases which, on becoming sick, left the colony for their homes in the country.

This is a common practice in Hong Kong, where so many of the male population live in common lodging houses and keep their wives and children in Canton, or "Canton more far."

2. Those cases which were concealed ignorantly or deliberately by the Chinese practitioners.

As the Chinese are willing to pay large sums of money in order to avoid interference in their homes from sanitary officers, this might include a fairly large number of cases.

3. Mild cases which escaped detection. There is every reason to suppose that this included a very large number of cases.

(a) About this time many of the local doctors noticed that a large number of cases occurred in their practice, presenting features identical with mild cases of cerebro-spinal fever, *i.e.* headache, sickness, stiffness of neck, varying degrees of prostration and complaint of dazed sensation.

When the Chinese schools re-opened at the end of February, after the China New Year holidays, the headmistress of one of the girls' schools containing between 400-500 pupils, situated in a very crowded portion of the town, observed to me that several of the teachers had commented on the strange appearance of a number of the pupils. They looked ill and exhausted, but it was the peculiar dazed expression in the eyes that attracted attention. Upon enquiry they said they had been ill with fever, headache and vomiting. Further information was impossible to obtain as no doctor had attended, but it is probable that these may have been mild cases of this disease. This peculiar dazed expression in the eyes of Chinese convalescents was commented on by

several observers, and was particularly noticeable in contrast to the usual unfathomable fixed oriental stare.

(b) At the local British Medical Association meeting in March, called to discuss the epidemic, the Hon. Secretary, Dr Marriott, stated that he had about an average of ten cases a day presenting the following features in varying severity—headache, stiffness of neck, dazed sensation, slight fever and vomiting. These rendered the patient more or less prostrate, but in a few days recovery took place and there were no after effects. Several other members gave similar experiences, but as no routine examination of the cerebro-spinal fluid had been made and no naso-pharyngeal swab taken, no conclusion was reached as to the nature of the diagnosis.

(c) It is quite impossible to estimate the number of the mild and missed cases, but it is reasonable to assume that they form a not inconsiderable number, which, if added to the number of known cases would lessen the percentage mortality in this epidemic.

Age and sex incidence. Out of a total of 1040 cases, with a mortality of 85.48 per cent., 635, or 61 per cent., were found to be males, 541 of whom died, giving a male mortality of 85.19 per cent., and 405 females, 348 of whom died, giving a similar female mortality of 85.92 per cent.

Sophian points out that Dr Steiner, State Medical Officer for Texas, obtained somewhat similar figures in an epidemic of cerebro-spinal fever, where, out of 2,575 cases, the number of males affected was 1595, or 62 per cent.¹

For the purpose of this paper the cases have been arranged in age periods of five years.

It will be seen from Chart No. 1, p. 294, which is an analysis of 1040 cases to show age and sex incidence and mortality that the largest number of cases, viz. 318, or 30.5 per cent., of the total number of cases, occurred in children of five years and under, with the high mortality of 307, or 96.5 per cent. At this age period the male cases are slightly in excess of the female.

In the second age period, from six to ten years, the number of cases falls to 123, or 11.8 per cent. of the total number.

It further falls to 106 in the following age period of 11–15 years, that is 10.19 per cent. of the total cases, and the number of male and female cases in these two age periods are about equal.

In the reports of the New York epidemic of 1905 6–7 the number of children under ten years affected is said to be 67 per cent., 65 per cent. and 68 per cent. of the total cases affected in these three respective years. In Hong Kong the returns for less than four months show that 441 cases, or 42.40 per cent. of the total cases occurred among children under ten years.

In the 16–20 period the number rises slightly to 111 cases, or 10.6 per cent. of the total. This number is made up of 90 male cases, 21 female cases, as shown in Chart No. 1, where the lines representing the numbers of male and female cases diverge widely at this point.

¹ Sophian. *Epidemic Cerebro-spinal Meningitis*, 1912.

The number of cases gradually diminishes during the following age periods until that of 66-70 years, where it is represented by three males with a mortality of 100 per cent.

It has been frequently stated that cerebro-spinal fever is a disease to which young adult males are peculiarly susceptible and at first glance it would appear to be borne out by the figures in Hong Kong (Chart No. 1). It is a well-known fact that the Chinese population in Hong Kong is composed largely of males. In the last census taken in Hong Kong in the year 1911, it was found that Chinese males over 15 years formed 54 per cent. of the total Chinese population, and on comparing the numbers in the census table given for males and females

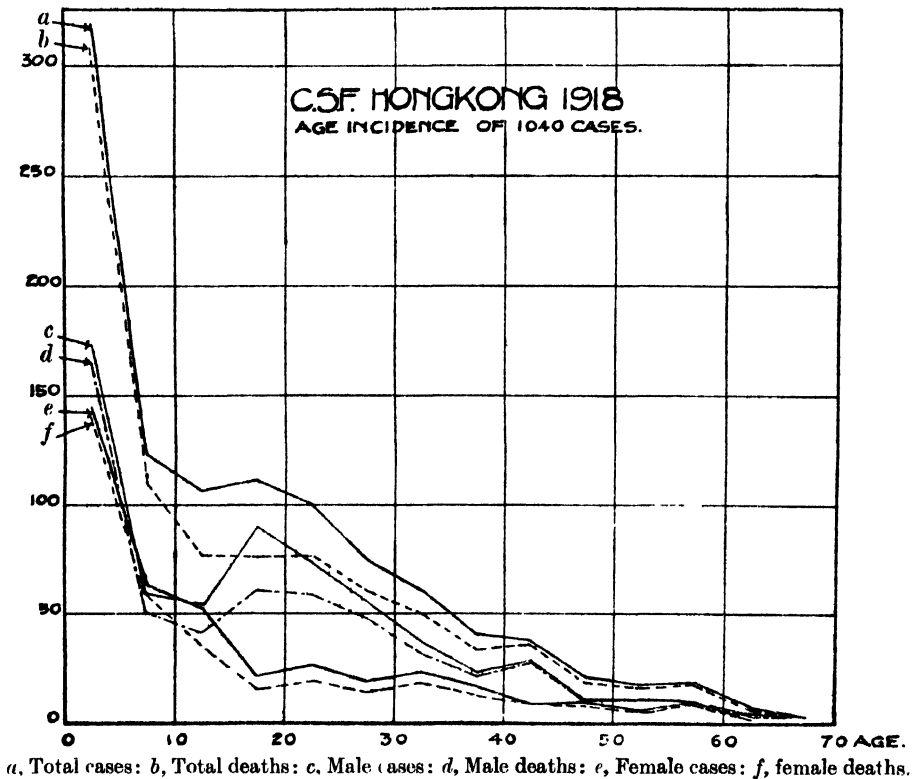


Chart 1. Graph showing mortality, age and sex incidence in 1040 cases.

during the respective age periods it is found that for the age period 16-20 years there are 31,290 males and only 12,913 females. It would naturally be expected that there would be more cases of cerebro-spinal fever among the larger number of males at this age than among the smaller number of females.

The census for 1911 is the nearest approach to accuracy obtainable, but only a census taken at the actual time of the epidemic could be held to be accurate, owing to the migratory nature of the population in Hong Kong.

In Chart No. 2 the age incidence of the total cases has been represented in percentage of the population as given by the last census taken in 1911. In

this chart the susceptibility for adult males of 16–20 years as shown by the peak in Chart No. 1 is no longer evident. It is wiped out when the peculiar local conditions as to population are taken into account. From 55–60 years there is apparently a rise in susceptibility more especially in the female, but the number of cases which occurred at this age is so small that no generalisation can be drawn beyond the one that susceptibility increases slightly with old age. The curve representing male and female cases in Chart No. 2, cross and recross and nothing definite as to sex incidence can be deduced.

It would appear then, taking both these charts into account, that under five years is the most susceptible age, after which the susceptibility drops 38 per cent. and diminishes gradually to old age, when it again shows a slight

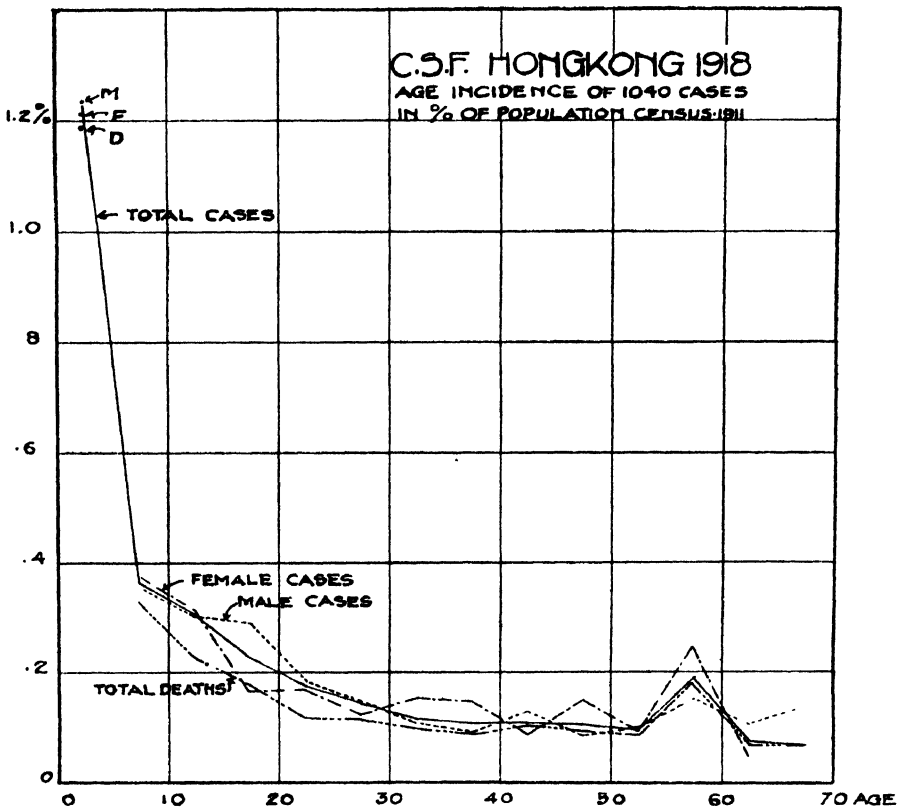


Chart 2 Graph showing mortality, age and sex incidence in 1040 cases represented in percentage composition of the population according to the census of 1911.

increase. Speaking generally the younger the individual, the greater the susceptibility with the added proviso that the extremes of life suffer most.

Incidence among Europeans. Out of 1040 cases only four European cases were notified between the onset of the epidemic and June 1st, three of which proved fatal. About ten days after the epidemic was recognised the first European case occurred in a middle aged business man in a debilitated state of health who succumbed to the attack in about three days.

Two English ladies, also in a low state of health, formed the second and third European cases, separated in time by a few weeks. The relatives of these two patients were strongly of the opinion that infection was the direct result of a shopping expedition to the Chinese part of the town undertaken two days previous to the attack. Both cases proved fatal after a very brief illness.

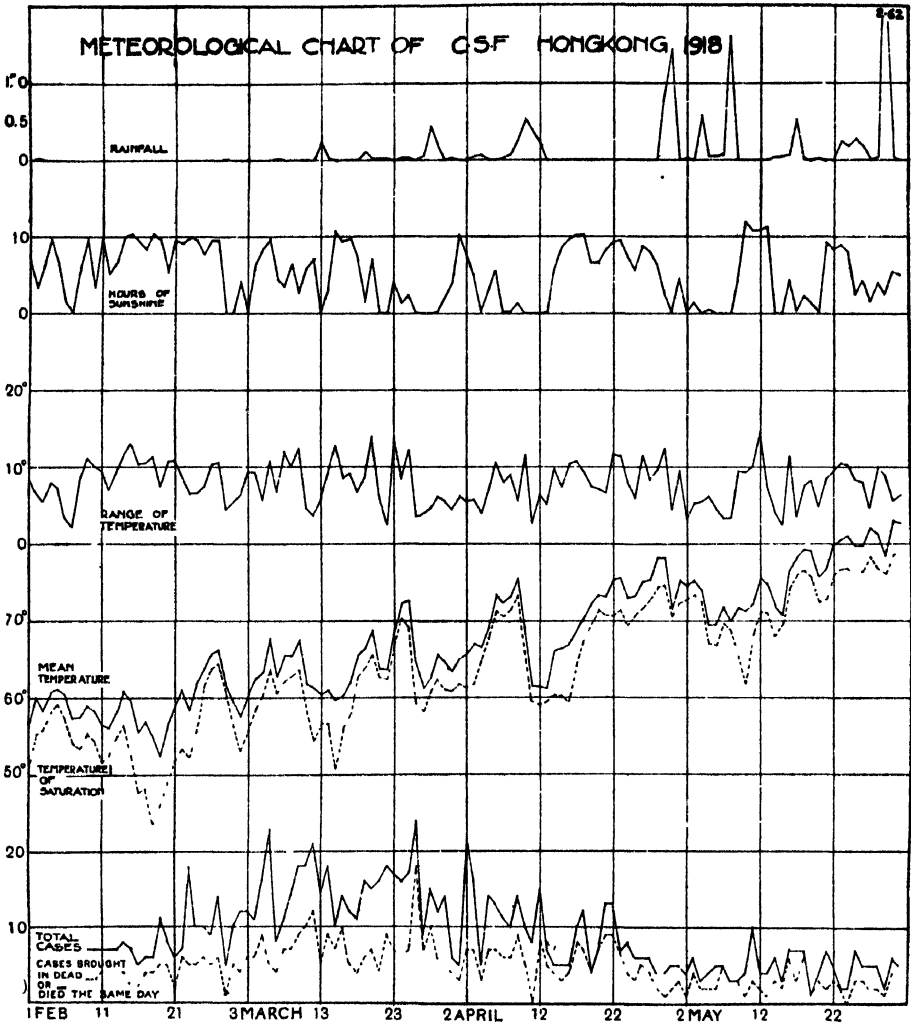


Chart 3. Showing relationship of daily number of cases of cerebro-spinal fever to the meteorological conditions.

The fourth case was that of an English schoolboy who fell ill during the holidays. He lived in a European Hotel and several members of his family and several of the Chinese servants were afterwards found to be carriers. After a protracted illness he recovered.

III. METEOROLOGICAL CONDITIONS.

The view that the onset of the disease was affected in some way by the meteorological conditions has been held by many observers, but the conclusions reached have been of a widely divergent nature.

Foster and Gaskell¹ write as follows: "Among the cases with which we had to deal in this outbreak, there appeared to be a certain relationship between a rapid fall of the barometer and the onset of the disease.... The conclusion may therefore be drawn that bad weather conditions alone are not the chief predisposing cause; rapid daily variations of temperature with or without much rain are of far greater importance."

Worster-Drought and Miles Kennedy² state that "There appears to be some connexion between diminished rainfall and an increase in the case incidence of the disease," and again, "There is no apparent relation between low atmospheric temperatures and cases actually suffering from the disease."

Compton³ is of the opinion that "Cerebro-spinal fever is a weather disease, outbursts being associated with sudden saturation of the atmosphere by water vapour combined with equable conditions of temperature—the meningococcus being about."

In Hong Kong, owing to the size and localised nature of the epidemic and the existence of an observatory from which accurate meteorological data could be obtained, an excellent opportunity was afforded for obtaining material on which reliable conclusions could be based. I have to thank Mr Claxton, Director of the Royal Observatory, Hong Kong, for furnishing early information of all meteorological returns.

With regard to the meteorological conditions in Hong Kong and their influence on the course of the epidemic, it would appear from analysis of the charts that once the epidemic had started, temperature had the most important influence on its course. It is observed that the curve of the daily incidence of cases follows a remarkably wavy course. The four highest peaks of this curve are sharp, and in each case represent an incidence of over 20 cases per day. Upon investigating the weather charts to ascertain if any connexion existed, it was found that certain meteorological conditions were constant for each one of these four peaks.

1. Looking at the first of the high peaks which occurred on March 6th and represented 23 cases, it was observed that on the fourth, fifth, and sixth days before this date the mean temperature showed a drop of 9° F. over three days, or 3° drop per day. On these three days the mean wet and dry bulb temperature curves approach each other, registering 2°, 3°, and 4°, difference respectively, showing that with the falling temperature the air was very moist. This was associated with a low range of temperature of 6°, 5°, 4°, per day respectively, and a complete absence of sunshine on the fifth

¹ *Cerebro-spinal Fever*, pp. 129-130.

² *Cerebro-spinal Fever*, 1918.

³ *Lancet*, 1917, xi. 15.

and sixth days and four and a half hours on the fourth day. No rainfall was recorded and the mean wet bulb temperature showed a drop of $11\frac{1}{2}^{\circ}$ F. over the three days.

2. Taking the next peak in order, 21 cases, which occurred on March 12th, it is observed that five days before this there was a drop in the mean temperature of 5° F., that the difference between the mean wet and dry bulb temperature registered only 2° F. The range in temperature dropped from 11° to 6° , and the hours of sunshine from nine and a half to four hours.

3. Coming to the third and highest peak of all, which occurred on March 26th, representing a total of 24 cases (18 of which were brought in dead, or died the same day), it is observed that five days previous to this the mean temperature dropped 5° F., the mean wet bulb temperature dropped 3° F. and remained at this temperature for two days, the fourth and fifth day before the peak, and during this time the difference between the mean wet and dry bulb temperature was only 1° F. On these same two days the range of temperature had dropped to 6° and 3° respectively and there was no sunshine registered in the colony. Rain fell on these two days to the extent of about .05 inch.

4. With regard to the last of the four high peaks on April 2nd, when 21 cases (seven dead) were reported, it was noted that a drop of $3\frac{1}{2}^{\circ}$ and 8° F. in the mean temperature, and 1° , and 10° F. drop in the mean wet bulb temperature occurred on the sixth and seventh day previous to the peak. The difference between the mean wet and dry bulb temperature was $2\frac{1}{2}^{\circ}$ on the sixth day and 5° on the seventh day. The range of temperature was 3° on the sixth day and 4° on the seventh day, and the hours of sunshine were nil on the sixth and seventh days respectively. The rainfall on these two days was also nil.

By comparing the charts further it is evident that practically every peak in the case incidence chart is represented by a corresponding depression on the temperature chart, ante-dated by a few days. This is specially noticeable towards the latter end of the epidemic when, on April 9th and 10th the mean temperature and the mean wet bulb temperature dropped 14° F. over the two days, approximating to within 2 degrees of each other and remained at this—for the time of the year—low temperature for three days following, viz. April 11th, 12th and 13th, accompanied by a complete absence of sunshine, with the result that on April 17th and 18th the incidence of cases rose from the previous average of five daily to that of 10 and 12 a day, forming the peak shown on the curve on April 18th.

A similar instance is noted on May 11th, when 10 cases were reported after a week in which the highest daily number did not rise above five cases. This was associated with a drop in the mean dry and wet bulb temperature, saturation of the air as shown by the approximation of these two temperature curves, low range of temperature and practical absence of sunshine on the fifth, sixth and seventh days previously, viz. May 4th, 5th and 6th. This interval or lag is thus shown to increase as the epidemic progresses and varied from three to four days in February to five, to six, to seven days in April and May.

Conclusions to be drawn as to the influence of meteorological conditions. It is evident from this analysis that:

1. Temperature showed the most influence. A drop in the temperature being invariably followed after a lag of a few days, by a rise in the number of cases. This lag tends to increase from three to four days at the beginning to six to seven days at the end of the epidemic.

2. That this fall in temperature was found to be associated with the following conditions:

(a) Steady maintenance of the low temperature as shown by the low range of temperature.

(b) Saturated condition of the air as shown by the approximation to each other of the wet and dry bulb temperatures.

(c) Absence of or diminution in the amount of sunshine.

3. That the rainfall showed no appreciable effect.

IV. GEOGRAPHICAL DISTRIBUTION AND OVERCROWDING.

The island of Hong Kong has an area of about 32 square miles and the city of Victoria situated on the northern side of the island has a frontage on the sea of nearly five miles and is separated from the Kowloon portion of the colony by the harbour (Map I).

That portion of the mainland between the shore and the Kowloon Hills known as Kowloon, has a seaboard of about 13 miles and an area of about 16 square miles (Map II, p. 300).

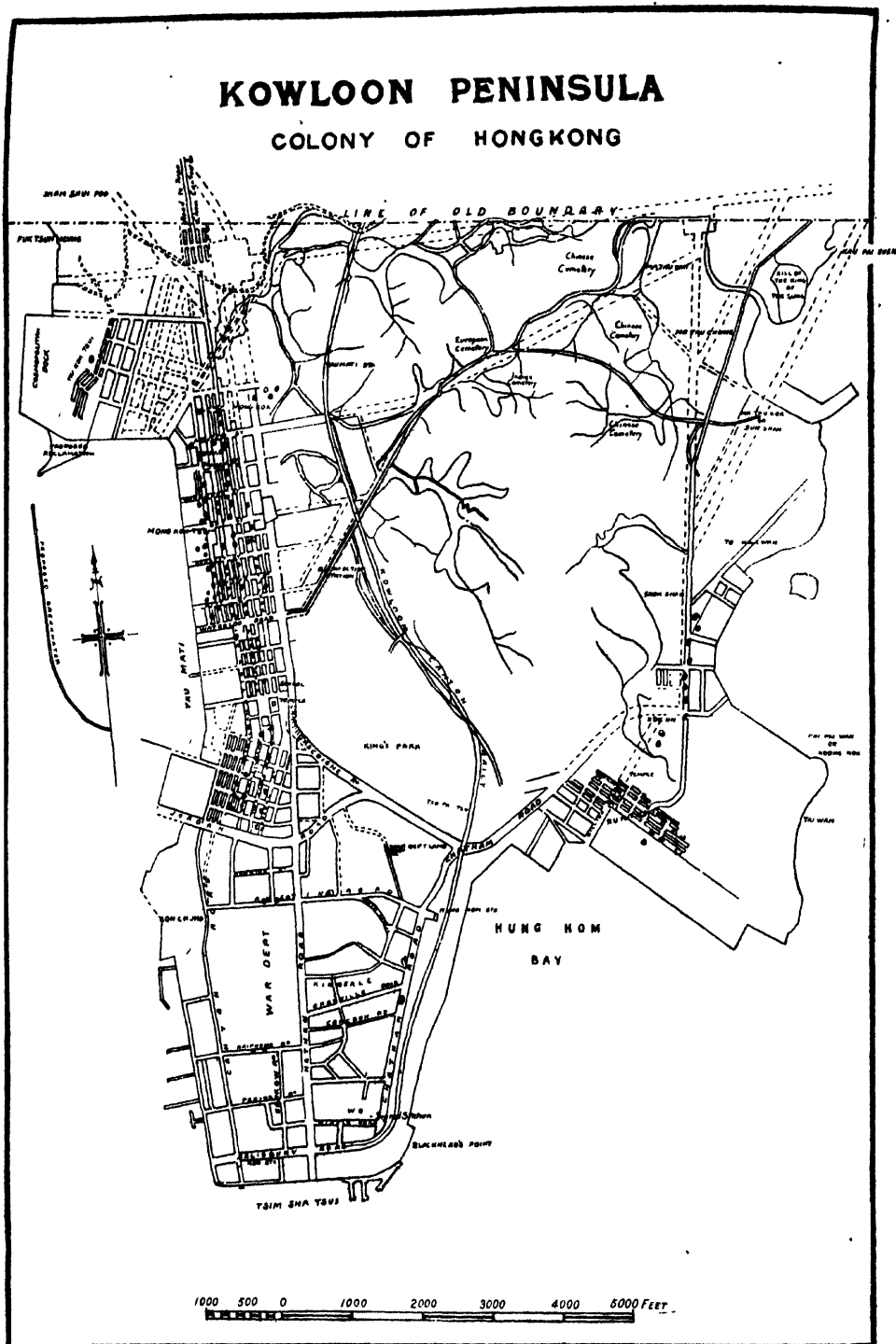
In the Annual Report of the sanitary board for 1917 the distribution of the population estimated to the middle of 1917 was as follows:

Non-Chinese civil population	...	13,500
Chinese civil population:		
City of Victoria (including Peak)	280,700	
Villages of Hong Kong	... 15,300	
Kowloon (including New Kowloon)	77,200	
New territories (land)	... 89,900	
Population afloat	... 58,500	
Total Chinese population	... ———	521,600
Total civil population	535,100

The following comment was appended to this table:

These figures have been estimated by the usual method based on natural increase as shown by the Census returns of 1906 and 1911 to which the number 10,000 has been added to allow for the influx of Chinese due to disturbances in Keong-tung Province.

There is no means of estimating the number of Chinese in the colony at any given time except by a Census and until a new Census is taken the present estimated figures must be considered to be quite unreliable and are in all probability much too low.



In the Sanitary Board Report for 1916 the head of the sanitary department states as follows:

The population has been so much disturbed by frequent immigration of Chinese refugees that too much reliance should not be placed on these figures. It is estimated that there were 120,000 such refugees in the colony at one period in 1916.

It is evident then that the total Chinese population of 521,600 as estimated for the year 1917 with a population of 280,700 estimated for the city of Victoria is much too low. More especially is this the case for Victoria where, in certain portions of the town, the streets, lanes and houses are so filled with people in the day time that it seems almost impossible to find a passage way.

The built over area in the city of Victoria is stated in the Sanitary Report for 1916 to be 802 acres which taking the population at 280,700 as estimated for the year 1917 would give an average density of 350 persons per acre.

It is stated in this report for 1917 that there are in the city of Victoria 9921 Chinese houses with an average of 3.1 floors per house. As the Chinese population for the city of Victoria is estimated for the year 1917 at 280,700 this would give an average of 28.8 persons per Chinese house. The average number of floors being 3.1 per Chinese house, this works out an average of nine persons per floor in each Chinese house.

In the Sanitary Report of 1916 a table is given showing the acreage of the city health districts with the houses and civil population estimated for each district.

In this table these health districts which showed the greatest density were No. 9 with a density of 863, No. 6 with a density of 981, and No. 5 with a density of 1000 persons per acre. A comparison with the state of affairs as recorded in the census year of 1911 is given in Table B.

Table B.

Year	Population, city of Victoria	Average number of persons per floor	Density per acre			
			Average	No. 9 H.D.	No. 6 H.D.	No. 5 H.D.
1910	178,300	6.9	252	578	615	693
1911	219,386	8.3	303	727	800	956
1916	270,300	8.7	352	863	981	1000
1917	280,700	9	350	No information		

Density per acre. In the Sanitary Report of 1911 compiled after the census was taken, it is stated that the population for the city of Victoria was 219,386, that the average number of persons per floor per Chinese house was 8.3; the average density for the town being estimated at 303 per acre.

Upon comparing the estimates for the years 1910 and 1911 as given in Table B, it is noted that the city of Victoria was growing out of all proportions to the estimated ratio of increase.

Thus in 1910 the average number of persons per acre was estimated at 252 and the following year it was found at the census to be actually 303 persons per acre, while the average number of persons per floor per Chinese

house had risen from 6.9 in 1910 to 8.3 in 1911. It is obvious from this that, prior to the last census, the authorities were in ignorance as to the extent of overcrowding then existing.

In these most overcrowded health districts in Victoria the extent of this ignorance is shown by comparison of the figures as given in Table B for the years 1910 and 1911, where the margin of error was in No. 9 Health District 149, and in No. 6 Health District 185, and in No. 5 Health District 263 persons per acre.

For the year 1917 the average number of persons per floor per Chinese house is estimated at nine, a figure which would mean an increase of about .7 persons per floor in the last six years.

As this calculation is based on the population estimates for the year, which are admittedly unreliable and much too low, the probability is that this figure should be considerably higher and that the next census will show an even more remarkable margin of error in the estimated figures than the last census.

If it be accepted that the "true index of density is the number of persons to each occupied room," and that "this test combined with a determination of the population in a given area would give the most trustworthy estimate of density¹," it is highly probable that Hong Kong, in the next census, will head the list for overcrowding in any British community.

Migration of the population. Migration is of considerable importance from the public health aspect in this epidemic.

Hong Kong is characterised by the large migratory section of the Chinese population which comes and goes practically unheeded. The migratory population is made up as follows:

1. Chinese emigrants leaving Hong Kong for ports other than China. The numbers are given in the Blue Book as follows:

In 1917 96,295.

Chinese emigrants returning to Hong Kong from ports other than China:

In 1917 98,232.

2. The known passenger traffic between Hong Kong and the mainland of China. The numbers are as given in the sanitary reports:

(a) For year 1917. By river steamer. Arrivals ... 870,837

Departures ... 844,480

(b) ,, ,, By rail. Arrivals ... 352,008

Departures ... 309,394.

This number includes some European traffic, but the number is negligible in comparison with the Chinese traffic.

3. Unknown passenger traffic between Hong Kong and China. This is, even in war time, an unknown quantity, as junks and sampans containing entire families of three generations arrive and leave Hong Kong daily unhampered.

Adding together these figures, it is found that 1,321,077 people arrived in

¹ *Vital Statistics*, by Sir A. Newsholme.

Hong Kong and 1,250,172 people departed from Hong Kong during the year 1917.

These figures are mainly Chinese, but include some little European traffic on the river steamers and railway, but do not include the passenger traffic on 750 ocean-going steamers stated, in the Blue Book for 1917, to have visited the colony at varying intervals of time during the year.

This large number of new entries into the colony, introducing, as it does, fresh susceptible material for the spread of infection, has a considerable bearing on the extent of the epidemic, as will be shown later.

The migratory nature of the population no doubt plays an important part in the dissemination of the disease, for in March an outbreak of 60 cases occurred at Swatow, a neighbouring coast port about a day's journey from Hong Kong. The first case which occurred in Shanghai was a European who had been to Hong Kong for race week at the end of February, and developed cerebro-spinal fever shortly after his return to Shanghai. A few cases only occurred in Shanghai, but it was well known that there was a big attack in Kobe, Osaka and Tokyo, although no official information was received from the Japanese.

It has been pointed out by Lieut. P. K. Olitsky in his report to the Hong Kong Government that the "entrance of a new susceptible element of population from a non-infected district into the epidemic area of the colony and this new element, usually coolies, existing in close contact with the disease, will tend to increase the number of cases: or they will cause a disturbance of the insusceptible ratio of the normal native population, and when the cold season approaches, or under other conditions favourable to the spread of epidemic meningitis, these will be new soil for an outbreak."

Dr Reynolds, the Medical Officer of Health for Canton, which lies on the West River, about 90 miles from Hong Kong, reported that there was no epidemic of cerebro-spinal fever there during the winter of 1918, and that the few cases which did occur were all recent arrivals from Hong Kong. This bears out experience in America where, during the epidemic in New York in 1904-1905, Philadelphia, less than 100 miles distant, was not attacked.

Housing conditions in Hong Kong. Some idea of conditions as they exist in Hong Kong may be obtained from the following quotations from old sanitary reports to the Government.

In the report on the question of the housing of the population of Hong Kong, by O. Chadwick, A.M.I.C.E., C.M.G., in 1902, he writes as follows:

"(1) The insanitary areas in Hong Kong have been formed by the crowding together of too many houses on too small a space.

(2) By sanitary defects in the design of dwelling houses.

(3) By overcrowding of the inhabitants of these houses. The crowding together of too many houses on too small a space has been effected by the construction of narrow streets and lanes and by the omission to provide adequate open space in the rear of houses in the shape of back yards and of back lanes. The houses have been brought into close proximity to one another instead of being well separated with ample space between them. The conditions vary in intensity according to the age of the built-over areas. The worst conditions are to be found where back-to-back houses have been constructed, or where the lane

between the rear of houses is not more than 6-8 ft. wide. In either case neither light nor ventilation is accessible from the back while only a very inadequate amount is obtainable from the narrow street or lane in front owing to the height of the houses being out of all proportion to the width of the street or lane. Similar unhealthy conditions occur when the rear of the houses abuts on the hillside with the additional circumstance that the house is rendered damp during the rains from percolation of water from the hill.

The Chinese tenement houses in Hong Kong differ in style from the European. They also differ from the ordinary Chinese houses in Canton or other Chinese city where the buildings are not more than two storeys in height, often not more than one. By some gradual process of evolution they have taken on the worst features of both kinds of houses and none of their best. The tenement houses in Hong Kong consist of several storeys, each storey containing one long room lighted at each end by a window but without lateral windows. Each room is subdivided by 6 ft. high partitions into cabins called cubicles, which accommodate an entire family. The room on each floor communicates in the rear by a bridge with the kitchen which is separated from the house by a small yard; and in front with a masonry verandah which encroaches on the public street and, which being separated by partitions from the adjoining houses is used as an additional room for the house.

The length of room without lateral windows, the kitchen buildings in the rear and the smallness of the back yard, by obstructing the free access of light and air, cause the two lower storeys at least to be dark and badly ventilated.

The verandahs in front still further increase this undesirable condition and the cubicles in the room intensify it to such an extent that none of the rooms are healthy habitations. The cubicle system leads to overcrowding in its worst form, and with the absence of light and fresh air, under its worst conditions, for with the existing design of buildings wherever there are more than two cubicles in a room, even in the upper storeys, the compartment is dark and devoid of fresh air. With darkness, absence of fresh air and overcrowding, it is impossible to keep them clean."

This condition of affairs, condemned by Mr Chadwick and Prof. Simpson in 1902, holds good to-day, but in an aggravated form, owing to the unknown extent to which the population has increased. An attempt was made in the early days of the epidemic to get permission roughly to estimate to what extent overcrowding did exist, by surprise night visits of the sanitary staff to the most overcrowded parts of the town, or even to those houses in which a case of cerebro-spinal fever has been reported. It was considered by the Government that this would annoy the Chinese community and permission was thereupon refused, and all idea of finding out the actual state of affairs had to be abandoned, except in so far as information could be obtained from the usual daily visits of the sanitary inspectors.

It was possible in 578 instances to get the number of persons said by the tenants to occupy the floor (or room) in the house in which the case occurred. The results have been tabulated as follows:

Number of inmates on one floor	No. of inspected floors	Percentage of total	No. of floors in which two cases occurred
1-5	116	20	3
6-10	154	26.6	11
11-15	156	26.9	10
16-20	97	16.7	11
21-25	40	6.9	5
26-30	9	1.5	0
31-35	6	1	0
578		99.6	40

From this it is found that 53·4 per cent. of the houses investigated of one floor and one room in which cases of cerebro-spinal fever occurred had more than ten occupants admitted to by the tenants. For the purpose of this table each child was calculated as one person, but it was quite exceptional for the tenants to admit that more than two or three children slept on the premises, and any excess over this number in the rooms were said to be children of friends on a visit.

Acting on the supposition that the above figures are accurate in so far as they do not overstate the case, it is found that 97 floors, or 16·7 per cent. of the available estimated floors contained from 16–20 inhabitants per floor, and that 40 floors, or 6·9 per cent. contained 21–25 inhabitants per floor.

Those houses which contained from 26–35 inhabitants were matchsheds, or else converted godowns, subdivided into a large number of small cabins, in which, although the overcrowding was serious, yet the ventilation and lighting were distinctly superior to that of the smaller houses, which may account for the fact that no double cases occurred in these houses.

Out of these 576 floors it was found that 40 floors, or 6·92 per cent. gave double cases, and that 18 out of these 40 floors (or 45 per cent.) of the houses giving double cases were situated in No. 9 health district. In addition, in five other instances, three cases came from the same address, but on investigation it was found that four of these addresses were matchsheds, temporary structures erected mainly for housing large numbers of coolies engaged on building operations. The remaining instance was that of a Chinaman who, with his wife and five children, arrived in the colony on February 15th and went to live under fair housing conditions in No. 4 health district, in a respectable Chinese boarding-house from which no previous case of cerebro-spinal fever had been reported. During the earthquake which occurred in Hong Kong on February 15th, he and his family all rushed with others out of the building into the streets, which were packed with frightened Chinese. Two days later, on the 17th, the Chinaman and his daughter, a sickly child, were removed to a private hospital and both died there on February 19th of cerebro-spinal fever. The nature of the illness was not detected during life. The wife of this man, who had remained with her husband until he died, was removed to a Government hospital on February 21st, and she died therein of cerebro-spinal fever on February 24th, 1918. Two cases were reported from the first floor of No. 12 First Street, in No. 9 Health District, which measured 38 ft. in length, 13·3 ft. in width and 12 ft. high, but contained no wooden cubicles and was occupied by 23 people including three children. That is to say, there was an allowance of 21·9 sq. ft. of floor area per person, counting each child as one, or of 23·5 sq. ft. of floor area counting each child as one half an adult allowance. As the Public Health Ordinance, Hong Kong, provides that 30 sq. ft. of floor area is the minimum allowance per head where no cubicles exist, and 50 sq. ft. where cubicles exist, this case is an obvious infraction of the law.

In a similar case in No. 9 Health District at 20 Fish Street, third floor, where

three cubicles without windows and two legal rooms or cubicles with windows existed, the total measurements of the floor were 39 ft. 4 ins. by 13 ft. 6 ins. by 17 ft. 6 ins. high. This floor supplied two cases of cerebro-spinal fever and was admitted by the tenant to contain 20 inmates, four of whom were children. The floor area works out at 26.5 sq. ft. per head, counting each child as an adult, or 29.4 sq. ft. per head, counting two children as one adult. But even on discovery of this gross infraction of the law the sanitary department was unable to take any action in the matter.

It is the custom among the Chinese to bolt every window and lock every door, as a precaution against thieves, and as there is no fireplace in a Chinese house, except in the kitchen, there is an absolute lack of ventilation and the consequent foul state of the air is indescribable.

An attempt was made to estimate the influence of the number of cubicles in a house on the case incidence during the epidemic, but no information of any value was elicited. It was found that:

Houses with 1 cubicle gave	17.6 per cent. of the total cases.			
„ 2 cubicles gave	21.5	„	„	„
„ 3 „ „	21.2	„	„	„
„ 4 „ „	12.3	„	„	„
„ 5 „ „	9.3	„	„	„
„ 6 „ „	4.1	„	„	„
„ 7 „ „	1.0	„	„	„
„ 8 „ „	.6	„	„	„

For the remaining 12.4 per cent. there were no cubicles, or else no information.

The majority of domestic Chinese houses in Victoria have more than one cubicle. It would appear that the houses with one cubicle have no great advantage over those with two or three. Cubicles are pulled down one day and erected again the next, therefore no correct list of houses with the respective number of cubicles was available. It must not be forgotten that in a Chinese house, in addition to the cubicles, there are beds all down the free wall space, and often a second layer of bunks half way up to the ceiling, and that each of these bunks or beds may be screened by a sackcloth or rag curtain hanging on a string, and may contain a family of varying number.

Spot map (see p. 300). The spot map shows, even to the casual glance, that in this epidemic there was a tendency previously noted in other epidemics, for the cases to arrange themselves in groups. In the outskirts of the town the groups are smaller and more widely separated, and in the centre of the town the groups are larger and more closely associated. On comparing the number of spots, or cases, in a given area over those parts of the town in which the spots appear to be most closely packed, the following results were obtained:

In No. 9 Health District, taking an area of 500 ft. sq. south of Queen's Road, between Centre and Eastern Street, this was found to contain 43 cases, or

4.1 per cent. of the total number. In the similar area immediately adjoining, lying between Centre and Western Street, there were 25 cases, or 2.4 per cent. of the total number. In No. 6 Health District, in the area 500 ft. sq., north of Caine Road, between Pedder Street and Sing Wong Street, there were 27 cases, or 2.6 per cent. of the total. In No. 5 Health District, in the area 500 ft. sq., south of Hollywood Road, lying between Peel Street and Sing Wong Street, there were 31 cases, or 2.98 per cent. of the total number. In Wanchai and Kowloon the largest number of cases in any area of 500 ft. sq. was found to be 20, or 1.92 per cent. of the total number.

In No. 9 Health District, taking an area of 1000 ft. sq. lying between Eastern and Western Streets, extending from Des Voeux Road to High Street, it is found that 100 cases, or 9.6 per cent. of the total cases were resident in the streets included within these boundaries. Twelve of these cases came from six floors. Taking the next most crowded areas of the same size, situated partly in No. 6 Health District and No. 5 Health District, north of Caine Road, lying between Real Street and Ladder Street, there were found to be 80 cases, or 7.6 per cent. of the total. Four floors in this district gave six cases. In a similar area situated partly in No. 6 and No. 7 Health Districts, extending from Queen's Road to Caine Road, lying between Sai Street and Sing Wong Street, there were found to be 63 cases, or 6 per cent. of the total number. Four floors in this district also gave eight cases. In Wanchai, in No. 2 Health District, and other parts of the town where the cases were more widely separated the largest number of cases in a similar area works out at about 2.3 per cent. of the total.

In Kowloon, in Mong Kok Tsui, the number rises to 4 per cent. of the total, but neither in Kowloon, or in Wanchai did any one floor give more than one case. In one instance in each of these two districts two cases were reported from the same tenement but different floors.

The small Chinese hospital for children in High Street, No. 9 Health District, gave, in addition to many other cases traced to their homes, a record of 43 cases in which a wrong address had been given and no trace of the family could be obtained. No doubt many of these cases came from the houses in the immediate neighbourhood.

From these results it is seen that the area in No. 9 Health District lying between Eastern and Western Streets, more especially towards the Eastern Street end contained the largest number of cases. This district is said to have a density per acre of 727 in the 1911 sanitary report, which, being the report of the census year, is presumably reliable for that year. For the year 1916 the density is said to be 863, which is quite unreliable and for the before-mentioned reasons is probably much too low. In any case, however, No. 9 Health District is well known locally to be the most overcrowded portion of the colony.

This is borne out by the following incident. On one occasion during the course of the cerebro-spinal fever epidemic a number of influential Chinese visited the Sanitary Board Office, in order to see the spot map, and the spokes-

man of the company asked to see first, the area lying between Eastern and Western Streets. After some study of the map, this Chinese gentleman said, "Yes, it looks as if overcrowding had something to do with this disease, judging by the numbers of spots in First Street, Second Street, Bridges Street and Staunton Street." These streets were well known to him to be the most crowded portions of the colony.

It is interesting to note that the three health districts in Victoria, viz. Nos. 5, 6 and 9, found at the last census to be the most grossly overcrowded, yield in the epidemic of cerebro-spinal fever the largest number of cases. From the census returns, it would have been expected that the order of the maximum incidence of cerebro-spinal fever would have been, first, No. 5 Health District; second, No. 6 Health District, and third, No. 9 Health District, whereas it was found that the results were—first, No. 9 Health District, second, No. 5 Health District, and third, No. 6 Health District. But No. 9 Health District is popularly supposed to be the most overcrowded portion of the town and it supplied 9·6 per cent. of the total cases, and the highest number of houses giving more than one case, so it may reasonably be expected that the next census returns will show No. 9 Health District to possess the greatest density per acre of any of the health districts in Victoria.

The general conclusion to be drawn from a careful study of the Spot Map is that the number of cases of cerebro-spinal fever in any given area varied directly with the overcrowding in that area, and that this bears out the generally accepted statement that overcrowding is one of the most important features in the epidemiology of cerebro-spinal fever.

V. HABITS OF THE CHINESE.

The well-known habit of the Chinese to expectorate profusely and constantly indoors and outdoors may be considered to have some relationship to the spread of cerebro-spinal fever in the colony. Expectoration among the Chinese lower classes consists of clearing the naso-pharynx by way of the mouth. The Chinese, as a nation, do not use handkerchiefs and, from their point of view, do not require them.

Taking this in conjunction with the fondness of the Chinese for eating in the streets, at little cooking stalls, or hawker's kitchens, food which has been exposed for some time in the open air and served in dishes which are only very occasionally washed and then dried with a filthy towel, which may have been used for several other unhygienic purposes, it is obvious that there is ample opportunity for conveyance of infection by food and food utensils. In addition, there is much drying and sorting of food on the streets and pathways all through the city.

VI. LENGTH OF RESIDENCE IN THE COLONY.

At the time of onset and during the course of the epidemic, enquiry was made into the length of time each case of cerebro-spinal fever had been resident in the colony previous to the first day of illness. The following table is an analysis of the results:

Table C.

	Length of residence previous to onset of cerebro-spinal fever	No. of cases	Percentage of total cases per day	Percentage of total cases per week
First week	1 day	19	1.8	9.5
	2 days	23	2.2	
	3 "	32	3.0	
	4 "	9	.8	
	5 "	5	.4	
	6 "	4	.3	
	7 "	11	1.0	
	1-2 weeks	50	.68	4.8
	2-3 "	104	1.42	10.0
	3-4 "	142	1.9	13.6
	Over one month	567	—	54.5
	No information	74	—	7.1
		1040	—	99.5

From this analysis the very interesting result is obtained that out of the total number of cases 7.1 per cent. had been resident three days, or less, and 37.9 per cent. one month, or less, in the colony when attacked by the epidemic.

It would appear that case incidence was high among the recent arrivals in the colony. Thus 9.5 per cent. of the total number of cases had only been resident in the colony one week, or less, and 3 per cent. of the total only three days when attacked by the disease. The numbers fell during the second week, rose again in the third week to a little above the level of the first week, and then increased to 13.6 per cent. for the fourth week of residence. It is evident from these figures that susceptibility in recent arrivals is high for the first three days, reaching its maximum on the third day, that it then falls for three days, after which it rises gradually, until in the fourth week of residence it reaches the average of 1.9 per cent. of the total cases for each day of the week.

The very high case incidence occurring in arrivals of three days' standing may have some relation to the period of incubation, which, although generally accepted to be from one to five days, might be held to be one to three days, with a special predilection for the third day.

Flexner¹ states "that the unusual conditions surrounding the military life exert an influence at the outset is indicated by the circumstances that it is the fresh recruit and not the seasoned soldier who especially suffers from epidemic meningitis."

It would appear then that the fresh Chinese recruit to Kong Hong is also

¹ "Control of Meningitis," in *Journal of the American Medical Association*, Aug. 24th, 1918.

especially liable to suffer from cerebro-spinal fever, and this is interesting, as overcrowding in sleeping quarters is probably the only condition common to life in a military camp and Chinese social life as it exists in Hong Kong.

Fildes and Baker¹ point out that when a case of cerebro-spinal fever occurs it is because a susceptible person is in contact with a high proportion of carriers and that new entries are specially prone to this disease. They consider that this etiological factor is more important than youth. In Hong Kong the high case incidence among recent arrivals is probably the result of the ideal conditions for the transmission of the disease which existed at this time in the colony. These may be tabulated as follows:

1. The large number daily of known cases and problematical number of missed cases.
2. The high proportion of carriers which no doubt existed during the epidemic.
3. The extremely migratory nature of a large proportion of the population which would entail a large daily influx of susceptible individuals from epidemic free areas.
4. Meteorological conditions.
5. Gross overcrowding in sleeping quarters in damp, insanitary, unventilated houses.

VII. OCCUPATION INCIDENCE.

An attempt was made to ascertain the influence, if any, of occupation on the incidence of the disease and an analysis was made of 336 adult cases in which it was possible to obtain information. The results were as follows:

Table of occupation incidence: 336 cases.

Occupation	No. of cases	Percentage
Coolies	117	34.82
House servants ...	44	13.09
Cooks	21	6.25
Students	18	5.35
Hawkers	11	3.27
Carpenters	11	3.27
Constables	7	2.08
Various	107	31.89
	336	99.92

Under the heading of "Various" is included 45 occupations, not one of which was represented by more than four cases.

In spite of the large number of clerks and office boys in the colony, it was noticeable that only four cases out of 336 occurred in this section of the populace.

The coolie, or unskilled labourer, forms a large proportion of the population in Hong Kong and lives a life of hard muscular fatigue, for everything that is

¹ *Report upon the Seasonal Outbreak of Cerebro-spinal Fever in the Navy at Portsmouth, 1916-1917, Medical Research Committee, Special Report, series No. 17.*

required in the colony, furniture, coals, or building material, is carried to its destination on the shoulders of the coolie, even if that destination should be 1500 ft. up on the Peak. This, taken in conjunction with the fact that the coolie belongs to the poorest class and therefore lives in the worst houses, under the most overcrowded conditions, is probably sufficient explanation why the coolie class of the population should furnish 34.82 per cent. of the cases of known occupation.

The comparatively large number of house servants affected might be explained as follows:

House servants in better class houses occupy coolie quarters at the back of the house and the majority of them keep their families in the country. Some of them, however, keep their wives and families in cubicles in the overcrowded portions of the town, where they visit them by night.

Many comparatively well-off Chinese live under poor housing conditions and keep a servant, or slave girl, who is housed in a bed under the stairs, or some odd corner in the passage way.

Cooks and students furnish a somewhat unexpectedly large number of the cases. The cooks were, for the most part, engaged in work in Chinese restaurants, and the theory that infection might be conveyed by food, or feeding utensils, would appear to receive some support from this high case incidence in Hong Kong. The comparatively large number of Chinese students affected is somewhat surprising but might be explained by the habits and poverty of this class.

Speaking generally, this analysis of occupation incidence goes to prove that it is the poorest, most hard worked and worst housed classes of the population that suffer most, and that if the doubtful case of cooks be excepted, occupation *per se*, has very little direct bearing on the incidence of the disease.

VIII. CARRIER RELATION TO THE EPIDEMIC.

It is to be remembered that Hong Kong is completely isolated and absolutely without expert advice; that in war time it took over two months for a mail to reach England; that works of reference are few in number and that the existence of the Medical Research Association brochure on cerebro-spinal fever was unknown in the colony until the arrival of Lieut. P. K. Olitsky, the Rockefeller Expert, on May 5th, 1918.

During the course of the epidemic therefore, the colony had to rely on its own resources.

At the request of the Hong Kong Government, the Rockefeller Institute very kindly lent the services of Lieut. P. K. Olitsky, for three months, to advise more especially on the preparation of serum and vaccine. This expert, however, did not arrive until May, when the epidemic was on the decline.

As a result of his bacteriological investigations in Hong Kong, he found that 95 per cent. of the 59 patients he investigated were infected with the Parameningococci of Dopter or Type 1 of Gordon's classification.

Lieut. Olitsky, undertook a personal investigation into the carrier ratio of the prisoners in the Victoria Gaol, and he states his results as follows:

"In conclusion, the results of the swabbing of a number of the inmates of the Victoria Gaol show:

1. That a number (24.61 per cent. of 130 inmates) of carriers of organisms indistinguishable from meningococcus had been found.
2. That these carriers harbour practically all types of the meningococcus.
3. That 3 of the carriers harbour organisms indistinguishable from the type of those found in the epidemic. One of these inmates carries almost a pure culture of one of the types prevalent in the epidemic.
5. Finally, among 600 or more prisoners no case of cerebro-spinal fever developed."

It was decided soon after recognition of the epidemic that routine carrier search among the contacts was a practical impossibility, for the following reasons:

1. Recognition of the epidemic did not occur until the early phase of the epidemic was passed.
2. The bacteriological staff and material were inadequate to cope with the large numbers involved.
3. Great difficulty was foreseen in compelling, or persuading the Chinese to permit any such routine search.
4. Difficulties which would arise in dealing with the carriers when found.

To quote Dr Arkwright¹, "The conditions which made a search for carriers desirable was a possibility of their isolation during the early stages of an epidemic." No such possibility existed in Hong Kong, and no attempt at a routine carrier search was carried out. It was arranged, however, to make a routine search for carriers in contacts of European cases, and this was done in the four European cases notified.

It was felt that something would have to be done to try and reduce the possibility of large numbers of carriers among the contacts. As no means existed of separating carriers from non-carriers, it was determined to act upon the assumption that all the contacts in the sleeping quarters on the same floor and using the same kitchen utensils, would be considered to be carriers and a possible source of infection.

The possibility of establishing steam inhalation chambers for the treatment of all contacts was considered, but in view of the difficulty of persuading the Chinese to go to any such place, and the necessity for immediately instituting some form of treatment, a small cheap portable atomiser, easily handled, which could be made locally, was designed by my husband at my request, to enable each sanitary inspector to carry out treatment of contacts in any infected house. Within a fortnight from the onset of the epidemic the first atomiser was in the hands of an inspector, and a few days later an atomiser of the

¹ Report of Proceedings of Epidemiological Section of the Royal Society of Medicine, *B.M.J.*, v. 3 15.

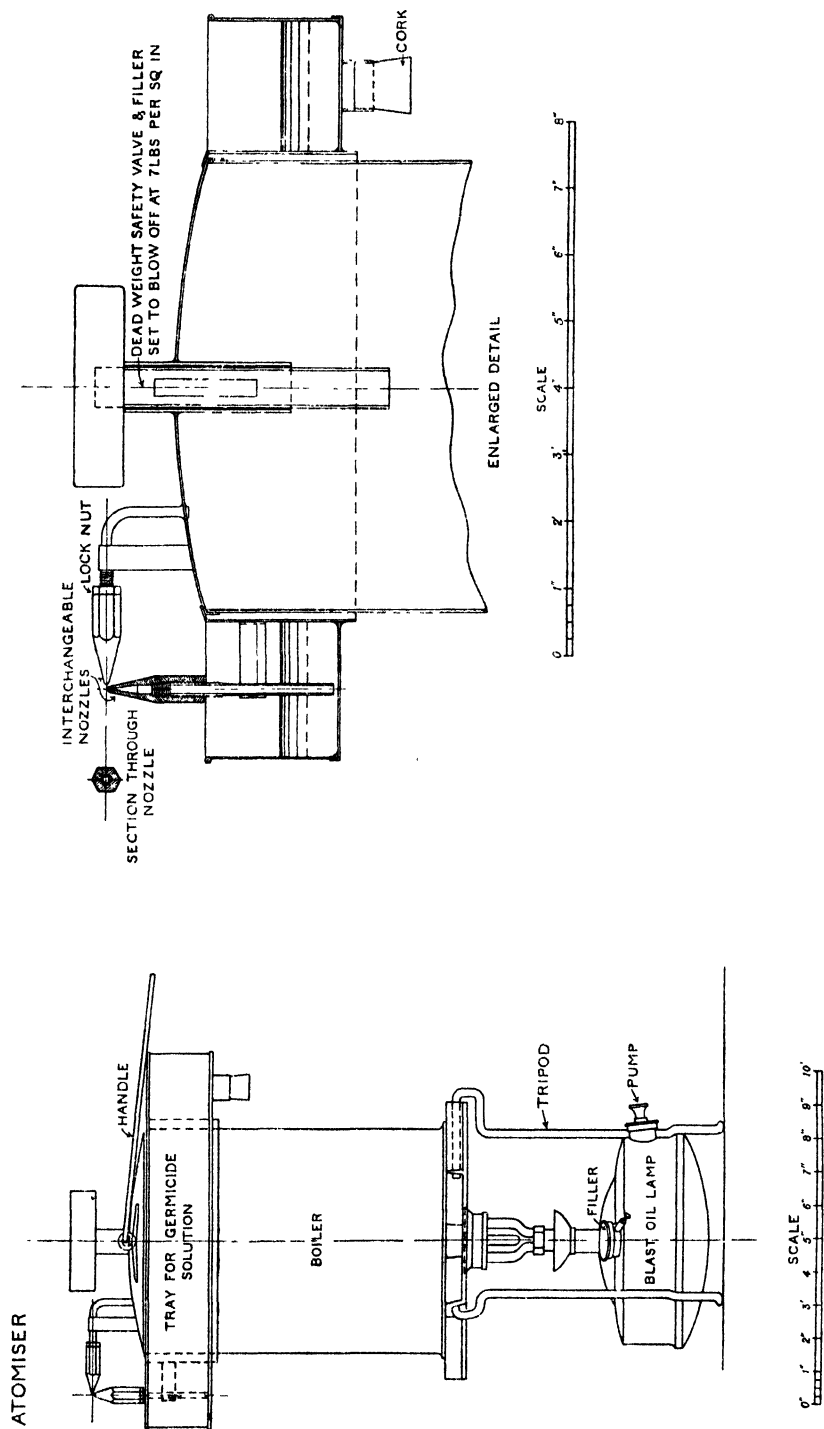


Fig. 1. Detailed scale drawing of construction of portable atomiser, designed by C. H. Gale for use in the Hong Kong epidemic.

improved design, as illustrated (see Fig. 1, p. 313), was in the possession of each of the 13 district sanitary inspectors.

As no chloramine-T could be obtained in the colony, it was a matter of necessity to use zinc sulphate in the atomisers. Zinc sulphate could be procured locally in large quantities, and had the additional virtue of being less unpleasant and irritating in use, a matter of some moment when it is remembered that the Chinese contacts had to be persuaded to take the treatment.

A rough-and-ready sort of inhalation chamber was devised in each house by setting the atomiser and its primus stove on a table inside the infected or other cubicle, and roofing in the cubicle with one of the Sanitary Board's canvas sheets. The average size of a cubicle is 8 ft. by 7 ft. by 6 ft., and an inspector, after a little experience, was able to manage the atomiser so as to ensure that one litre of 1 per cent. zinc sulphate solution was atomised in about 20 minutes, thus approaching as nearly as was possible to the instructions given for the use of Gordon and Flack's Falmouth Atomiser.

The family of the patient was treated first, and then all the remaining inhabitants of the room. As this had to be done during the day, it did not touch those who worked at any distance from their houses. Those who worked near were often fetched by their friends.

The inspectors called at each infected house daily for five days and treated each member of the household for five to ten minutes in the steam cubicle. The inspectors were instructed to pass each contact in turn in front of the atomiser and see that each one inhaled energetically while the steam played on the face. Some of the inspectors were more successful at this work than others and aroused so much interest in the Chinese that the inmates of the other floors in the infected tenement asked that they might also receive treatment.

In addition to this, a bottle containing the patent disinfectant Bacili-Kil solution was left at each infected house and the inmates instructed how to gargle and wash out the naso-pharynx. This disinfectant is an American patent production supposed to be equivalent to freshly prepared eusol solution electrolysed and rendered stable. It could be obtained at once in considerable quantity at a fairly cheap price and, being pleasant and non-irritating to use, was the most suitable preparation for the purpose which could be obtained locally.

Whenever it became known that atomisers were being used for treatment of contacts an insistent demand was made by the public that atomisers should be installed at various centres in the colony for general use.

The majority of the schools were closed from the time of onset of the epidemic till February 27th for the China New Year holidays. It was asked by the Sanitary Board that when re-opened, each of the large schools should be provided with an atomiser, and that the pupils should all undergo the treatment. This was done on the understanding that each school would be capable of undertaking the entire charge of the atomiser. This was entered into most enthusiastically by the schools, and only one accident occurred, in

which one child was scalded, owing to the bursting of the atomiser, as a result of the tying down of the safety valve by the responsible person.

It is impossible to judge whether this had any bearing on the course of the epidemic, but the fact remains that after the schools re-opened, only three cases of cerebro-spinal fever were reported among some 6000 scholars.

Public opinion in the colony became somewhat divided on the value of the atomisers for general use, but there can be no doubt they served the useful purpose of a panacea for the panic stricken. Owing to the widespread fear of infection from the unknown carrier, the Europeans and better class Chinese eagerly sought medical advice as to prophylaxis. The chemists did a roaring trade and pounds of nasal ointment and gargles were sold daily. Some of the local medical profession thought that the use of the atomisers encouraged throat irritation, and others that it cured the colds which were so prevalent during the entire winter.

Much of the irritation said to follow the treatment was, no doubt, due to over-indulgence; some of the people being atomised two to three times daily for several weeks. For a time, at any rate, a section of the colony went atomiser mad.

Atomisers copied from the Sanitary Board pattern (see Fig. 1), were made privately by different firms and sold to the public, so that it may be taken for granted that a certain proportion of the public were most thoroughly and constantly atomised. Atomisers were in use at the Gaol, Police Court, and General Hospital for the use of the Staff.

During my voyage from Hong Kong to Canada in July, 1918, every person on board ship was obliged by Japanese Port regulations to spend five minutes daily in a zinc sulphate steam inhalation chamber. As the shade temperature was about 90° F. the heat of the chamber was most distressing, in spite of the steam used being drawn from the ship's steam pipes, and there were many complaints of faintness and headache. I found by experiment that it was possible, by sitting below the level of the steam nozzle, to escape these unpleasant sensations and, during the time spent daily in the steam chamber, I did not ever experience the taste of the zinc sulphate.

In Hong Kong, if the nozzle of the atomiser was correctly adjusted and inhalation took place while the steam was playing on the face, the metallic taste of zinc sulphate soon appeared and remained for some time afterwards.

As treating by steam atomised drugs is the object of treatment in the steam chamber, it is difficult to see what advantage the inhalation steam chamber possesses over the more simple method of inhalation from the steam atomisers.

It would appear that if there is any value in the inhalation of steam atomised drugs, the simple form of steam atomiser should prove as efficacious as the steam chamber. It has the additional value of being cheap, portable and easily handled by one man. It can be used on a coal or oil stove, gas, or electric heater, and can be manipulated by an unskilled operator.

Early in the epidemic the question of what was to be done with the carriers

in Hong Kong when they were discovered came up for much fruitless discussion, and His Excellency The Governor gave it as his opinion that Chinese carriers and cases could not be prevented from leaving the colony and that legal action in the matter was out of the question. As no routine carrier search was conducted and the number of known carriers bore no proportion to the number of cases, the question never became a pressing issue.

It was recommended by me at a special meeting of the local branch of the British Medical Association, called to discuss the epidemic early in March, 1918, that if the local medical profession would recommend their fearful patients, more especially delicate women and children, to wear some form of mask or veil to act as a physical screen against droplet infection when passing through thickly populated Chinese districts, that this might prepare the minds of the public for a possible measure of protection against carriers. If known carriers became numerous and isolation and consequent interference with the business life of the community impracticable, it might be possible to enforce the wearing by the carrier of some form of physical screen which would prevent him acting as a source of infection, and enable him to carry on his usual vocation without endangering the public safety and to the great saving of the public money.

The manager of one of the business offices in Hong Kong, visited daily by hundreds of Chinese coolies, asked if in addition to the atomisers anything else could be done to safeguard the office staff, who were very alarmed at the prospect of possible carrier infection. It was suggested that a strip of transparent, closely woven muslin, 3 ft. high, should be stretched from end to end of the counter, to act as a screen between those on either side of the counter. This was erected, and the muslin dipped in disinfectant and washed daily and the fears of the staff were at once allayed.

The public panic was a most noticeable feature of the epidemic in Hong Kong. This was due almost entirely to fear of the unknown healthy carrier, and the knowledge that no steps were taken to ascertain the numbers, or identity of such carriers.

It would appear from the experience gained in Hong Kong that the recognition, and isolations of carriers whilst of great scientific interest played no part in the checking of the course of the epidemic wave.

It, moreover, became evident that any isolation of contacts and healthy carriers when found would be a practical impossibility owing to the large numbers involved and the financial difficulties even if the legal difficulties could be overcome and compulsion applied.

It remains then to be considered what steps, if any, should be taken in any future epidemic of considerable dimensions to deal with contacts and carriers. One question that arises is whether it is worth while, even, if practicable, to try to isolate contact carriers seeing that during an epidemic a large proportion of the general public have also been found to be carriers.

It is impossible to investigate the naso-pharynx of an entire population, and anything less would only be half measures.

It would seem then that the problem must be attacked from another direction, and that in the present position of our knowledge all that can be done is to render the carrier as harmless as possible. If coughing, sneezing and spraying from the mouth is the recognised mode by which infection spreads then it should be a simple matter to devise some form of protection against this infection.

A screen of transparent celluloid which is absolutely impervious to droplet spraying would meet the case, and if the milliner and hatter were consulted, a hat and screen in one, or a screen to fit the fashionable hat of the moment could be designed which would be efficacious and inoffensive. The screen would require to be a reasonable distance from the face to allow of the use of a handkerchief, and should extend from the hat brim to below the chin level in order to completely enclose the face. The contacts might be required to wear this screen until proved to be non-carriers, or failing this, for a given length of time, and the general public advised to protect themselves against possible infection by wearing it. If the epidemic were of serious dimensions the wearing of a screen might be enforced. In the case of a native population such as exists in Hong Kong this would be impracticable but employers could insist on employees wearing a screen during business hours and if Europeans wore a screen as a preventive measure the better class Chinese would soon follow suit and so a large section of the populace might be protected.

It would be at all events a step in the right direction, and once prejudice were overcome it would only be a question of time before the public would voluntarily use it during the course of an epidemic such as influenza or diphtheria, or any other disease suspected to be conveyed by droplet infection.

IX. SUMMARY OF CONCLUSIONS.

1. That there is reason to believe that sporadic cases of cerebro-spinal fever occurred in Hong Kong previous to the outbreak of the epidemic in 1918.
2. That the epidemic followed a widespread infection of influenza colds.
3. That the mortality out of a total of 1040 cases was 85.48 per cent.
4. That the most susceptible age was found to be the age period under five years.

The younger the individual, the greater the susceptibility, with the added proviso that the extremes of life suffered most.

5. That once the epidemic was established, a drop in the temperature, steadily maintained, accompanied by an increase in the saturation of the air as regards moisture, associated with a lack of sunshine, was found to be followed after a lag of a few days, by a rise in the number of cases. This lag showed a tendency to increase as the epidemic progressed, from three to four days at the beginning to six to seven days at the end of the epidemic.

6. That the number of cases in any given area varied directly with the overcrowding in that area, and this bears out the contention that overcrowding

is one of the most important features in the epidemiology of cerebro-spinal fever.

7. That the housing conditions in Hong Kong are such as to necessitate immediate action on the part of the Government, to safeguard the public health of the colony.

8. That new entries to Hong Kong formed a large proportion of the cases, and that this appears to be an important factor in the epidemiology of the disease.

9. That the poorest, most hard worked and badly housed portion of the community suffered most in the epidemic.

10. That the recognition and isolation of carriers is impracticable in an epidemic of any considerable dimensions.

11. That in the present condition of the knowledge of the means by which infection is spread, the wearing of a screen composed of some impervious material such as celluloid would seem to afford complete protection to the wearer against droplet infection.

12. That known carriers should be compelled to wear this screen until such time as they are proved free from the meningococcus. Thus protected, the carrier could pursue his usual avocation and the community be safeguarded from infection at a minimum of expense.

A COMPARATIVE STUDY OF BOVINE ABORTION AND UNDULANT FEVER, FROM THE BACTERIO- LOGICAL POINT OF VIEW.

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(With 2 charts.)

EARLY in 1914 Kennedy (1914) while testing some samples of goat's milk for agglutination of *B. melitensis*, found, to his surprise, that the control cow's milk gave a positive result. Following up this observation he noted that five out of the 13 specimens of cows' milk examined by him contained agglutinins for the organism of Malta fever.

Fabryan and Theobald Smith (1912) had shown that the tuberculous-like lesions produced by inoculating guinea-pigs with raw cow's milk were due to *B. abortus* Bang, the cause of bovine abortion. Since then other workers, notably Zwick and Krage (1913) confirmed the finding of *B. abortus* in the milk of infected cows, this excretion taking place irrespective of any lesion in the udder.

These facts remained uncorrelated until A. E. Evans (1918), in an illuminating piece of work, showed that *B. abortus* and *B. melitensis* were morphologically and serologically (agglutination) indistinguishable. Meyer, Shaw and Feusier (1920), later, corroborated Evans' views by a series of absorption tests.

It was for the purpose of further elucidating the relationship of these two organisms that the present investigation was undertaken.

The cultures used in this research were supplied by the National Collection of Type Cultures and are representative of strains isolated in America, on the Continent and in this country. In all, 13 strains of *B. melitensis*, 10 strains of *B. abortus* and 3 of *B. paramelitensis* were examined.

MORPHOLOGY AND NOMENCLATURE.

These three organisms are morphologically indistinguishable, occurring as small rods 3-5 μ in length with somewhat pointed ends. They are non-motile, stain uniformly with basic stains and are gram-negative. They are, however, somewhat pleomorphic and the same strain may show bacillary, cocco-bacillary or coccoid forms from time to time or the three forms may occur together in

the same culture. The age of the culture, the culture medium, and the method of staining have apparently nothing to do with this transformation.

The term "Micrococcus" is, therefore, inexact; and, though the term "Bacterium" would be correct, it would be better still if the generic name "Brucella," in honour of Sir David Bruce who discovered the first species (*melitensis*) in 1886, were adopted as suggested by Meyer and Feusier. Thus we would have *Brucella melitensis*, *Brucella abortus* and *Brucella paramelitensis*.

BIOCHEMICAL AND CULTURAL CHARACTERS.

The three organisms have the following common cultural and biochemical characters. They grow very slowly and scantily on ordinary agar, preferring glucose agar, on which medium they give a good growth after 36–72 hours' incubation at 37° C. The colonies are small, circular, with a smooth margin 2–3 mm. in diameter and whitish in colour. Later the growth tends to become confluent. In trypsin broth a uniform turbidity is produced after the third day without any surface pellicle. Litmus milk becomes alkaline and is not coagulated.

There is no change (acid or gas) in Hiss' serum with lactose, saccharose, dulcitol, mannitol or glucose, even after two weeks' incubation.

MODE OF INFECTION.

Infection with *B. abortus* may take place during copulation with males who have previously covered infected animals or who are themselves infected and excrete bacilli in their seminal fluid. The bedding may also carry infection, becoming contaminated with the vaginal discharges, amniotic fluid or foetal membranes from infected cows. Apparently the most usual mode of infection, however, is ingestion of food infected with amniotic fluid or afterbirth, as this ensures a considerably larger quantity of virus. After an incubation of 33–230 days a catarrhal condition of the genital passages with some discharge makes its appearance, and the secretion of milk is diminished. This is followed in 3–4 days by abortion accompanied by moderate pains and mild general manifestations. The animal recovers and then either remains sterile or aborts soon after service, generally after two months. The disease is highly infectious and rapidly spreads through whole herds affecting especially young animals. The males apparently act as carriers. The causal organism can be isolated from the spleen, liver, testes, seminal vesicles and uterine discharges as well as from the milk; the foetal membranes, stomach, amniotic fluid and cotyledons as a rule give pure cultures. Isolation and disinfection are very effective measures.

In undulant (Malta) fever, infection may occur through contamination of superficial scratches or pricks, though in the great majority of cases the disease is contracted by the ingestion of infected food—mainly goat's milk. It is well to mention, however, that cases have been reported in which infection was

produced by sexual intercourse, and in this respect it is worthy of note that enlargement of the testes occurs in a great proportion of the cases. The three cardinal signs of this disease are (i) great emaciation, (ii) evening sweats, and (iii) a prolonged, irregular fever continuing for weeks or even months. The disease is not essentially fatal and the mortality is only 2-5 per cent.

In goats the disease may be present without producing any obvious clinical manifestations.

In fatal cases the organism can be recovered from the blood (10 per cent.) from the spleen, liver, enlarged mesenteric glands, kidney, urine, saliva and milk.

Thus the two diseases present the picture of a bacteraemia, with a close similarity in the modes of infection, and of excretion of the causal organism.

IMMUNOLOGY.

Anti-sera were prepared against the three organisms, *B. abortus*, *B. melitensis* and *B. paramelitensis* and the 30 selected strains tested against these sera by means of the agglutination and absorption reactions.

Rabbits were employed in the production of the sera, receiving intravenous inoculations of killed 48-hour cultures (60° C. for 30 minutes). The first dose was 1500 millions and the second 3000 millions at a week's interval. One week after the last inoculation, the serum was tested, and if found satisfactory, the animal was bled out. A satisfactory serum gave complete sedimentation of the homologous strain in a dilution of at least 1 in 6100. Tubes were incubated for 3 hours at 37° C. and read after 12 hours' standing at room temperature.

The various cultures are referred to by their number only in the tables which follow, and for sake of brevity the findings obtained with nine only of the 30 strains are recorded. The results obtained with the other 21 strains differed in no respect from those given below.

Cultures of *B. melitensis*. Nos. 78, 80 and 893.

Cultures of *B. paramelitensis*. Nos. 82 and 84.

Cultures of *B. abortus*. Nos. 624, 830, 895 and 900.

Table I.

Anti-melitensis serum (titre 1 : 6400) tested against the various strains.

Strain	Dilution of serum								Control
	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12800	
78	+++	+++	+++	+++	+++	+++	+	-	-
80	+++	+++	+++	+++	+++	+++	+++	-	-
893	+++	+++	+++	+++	+++	+++	+++	-	-
82	+	-	-	-	-	-	-	-	-
84	+	-	-	-	-	-	-	-	-
624	+++	+++	+++	+++	+++	+++	++	-	-
830	+++	+++	+++	+++	+++	+++	++	-	-
895	+++	+++	+++	+++	+++	+++	++	-	-
900	+++	+++	+++	+++	+++	+++	+	-	-

Table II.

Anti-abortus serum (900) tested against the various strains.

Strain	Dilution of serum								Control
	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12800	
624	+++	+++	+++	+++	+++	+++	++	-	-
830	+++	+++	+++	+++	+++	+++	++	-	-
895	+++	+++	+++	+++	+++	+++	+++	-	-
900	+++	+++	+++	+++	+++	+++	+++	-	-
78	+++	+++	+++	+++	+++	++	-	-	-
80	+++	+++	+++	+++	+++	+++	-	-	-
893	+++	+++	+++	+++	+++	++	++	-	-
82	+++	+++	+++	+++	+	-	-	-	-
84	+++	+++	+++	+++	+++	+	-	-	-

Table III.

Anti-paramelitensis serum (84) tested against the various strains.

Strain	Dilution of strain								Control
	1 : 100	1 : 200	1 : 400	1 : 800	1 : 1600	1 : 3200	1 : 6400	1 : 12800	
82	+++	+++	+++	+++	+++	+++	++	-	-
84	+++	+++	+++	+++	+++	+++	++	-	-
78	++	+	-	-	-	-	-	-	-
80	++	++	-	-	-	-	-	-	-
893	+	-	-	-	-	-	-	-	-
624	+++	++	-	-	-	-	-	-	-
830	-	-	-	-	-	-	-	-	-
895	+	-	-	-	-	-	-	-	-
900	+	-	-	-	-	-	-	-	-

It will thus be seen that agglutination alone helps us little in arriving at any valid conclusions as to the serological relationship of *B. melitensis* and *B. abortus*, and a series of absorption tests was made, therefore, to determine this point.

Meyer, Shaw and Feusier (1920), in their paper quoted above, divided the organisms of undulant fever and cattle abortion into four groups serologically, their Group IV containing *B. paramelitensis* only. The results of the following absorption tests, however, do not lend support to this method of classification and justification is therefore felt in mentioning them in some detail.

TECHNIQUE OF ABSORPTION USED.

Antigens. The 48-hours' growth on 1 per cent. glucose agar slopes was washed off with sterile distilled water, using 0.5 c.c. for each culture.

The antisera, prepared as above were diluted 1 : 25 with salt solution (0.75 per cent.).

To the emulsion obtained from several slopes was added an equal volume of the diluted serum, giving a final dilution of 1 : 50 of the serum. The mixture was then incubated for two hours, centrifuged and the clear serum pipetted off.

The absorption was considered as satisfactory when all agglutinins for the absorbing strain had been removed.

It may be mentioned that broadly speaking, a full agar slope is practically always found sufficient to absorb all the specific agglutinins from 1 c.c. of 1 : 50 serum whose titre is 1 : 6400 (or less) in 2 hours. This obviates the subsequent absorption which is liable to become necessary if a smaller amount of organisms is used.

The technique of the agglutination experiments performed with the absorbed sera was the same as that already described earlier in this communication.

Only the more important results are recorded.

Table IV.

Anti-melitensis serum absorbed with *B. melitensis* (80) and tested against strains 80, 893, 830, 895 and 900.

Strain	Dilution of serum				Control
	1 : 100	1 : 200	1 : 400	1 : 800	
80	—	—	—	—	—
893	—	—	—	—	—
830	—	—	—	—	—
895	—	—	—	—	—
900	—	—	—	—	—

The same result was obtained after absorption with any other strain of *B. melitensis*.

Table V.

Anti-melitensis serum (80) absorbed with *B. abortus* (900) and tested against strains 80, 893, 900, 895 and 830.

Strain	Dilution of serum				Control
	1 : 100	1 : 200	1 : 400	1 : 800	
80	—	—	—	—	—
893	—	—	—	—	—
900	—	—	—	—	—
895	—	—	—	—	—
830	—	—	—	—	—

The same result was obtained after absorption with any other strain of *B. abortus*.

Table VI.

Anti-abortus serum (900) absorbed with strain 900 and tested against strains 78, 80, 893, 830, 895 and 900.

Strain	Dilution of serum				Control
	1 : 100	1 : 200	1 : 400	1 : 800	
78	—	—	—	—	—
80	—	—	—	—	—
893	—	—	—	—	—
830	—	—	—	—	—
895	—	—	—	—	—
900	—	—	—	—	—

The same results were obtained if *abortus* strains other than the homologous were employed or other anti-abortus sera used.

Table VII.

Anti-abortion serum (900) absorbed with *B. melitensis* (80) and tested against strains 78, 80, 893, 830, 895 and 900.

Strain	Dilution of serum				Control
	1 : 100	1 : 200	1 : 400	1 : 800	
78	-	-	-	-	-
80	-	-	-	-	-
893	++	+	-	-	-
830	+++	+++	+++	+++	-
895	+++	+++	+++	+++	-
900	+++	+++	+++	+++	-

Other anti-abortion sera absorbed with this as well as other strains of *B. melitensis* gave similar results.

The salient features of these absorption tests can be summarised as follows:

1. When an anti-melitensis serum is absorbed with *B. melitensis* all agglutinins for *B. melitensis* and *B. abortus* are removed.

2. The same result is obtained if *B. abortus* is used to absorb an anti-melitensis serum.

3. Anti-abortion serum absorbed with any *abortus* strain loses all agglutinins for both *B. melitensis* and *B. abortus*.

4. Anti-abortion serum absorbed with *B. melitensis* has lost its power to agglutinate *B. melitensis* strains but still agglutinates *B. abortus* to full titre.

From these results it would appear that *B. melitensis* is a sub-strain of *B. abortus*, in the sense employed by Schütze (1922).

Absorption experiments on the same lines with *B. paramelitensis* have not so far been carried out.

PATHOGENICITY.

The close morphological, bio-chemical and serological relationship between *B. melitensis* and *B. abortus* at once raises the question of their relative pathogenicity. This becomes much the more important if we consider the fact that 25 per cent. of the milch cows in this country are infected with *B. abortus*, and this percentage is even higher on the Continent and in America. These bacilli are found even in the "certified milk." A series of animal experiments on guinea-pigs, goats and monkeys was carried out, a few of which will be mentioned.

Guinea-pig 4. Intraperitoneal inoculation of $\frac{1}{4}$ -slope culture (48 hrs.) of *B. abortus* (900), 17. ii. 21. In 24 hours the temperature began to rise and reached 104° in 36 hours. The fever continued for three weeks, reaching its highest point (105° F.) at the end of the second week. The thermometer gave a higher reading in the evening (5 p.m.) than in the morning (10 a.m.). The animal looked sluggish and was disinclined to feed during the first week after injection. It was killed on March 16th.

Post-mortem. No marked congestion or exudate in peritoneum. Spleen enlarged and adherent to diaphragm with a small abscess between superior border and the diaphragm. Liver slightly congested. Kidney normal; testes and epididymis normal. Lumbar and mesenteric glands enlarged. No change was noted in heart or lungs. The chief histological finding was an increase of lymphoid tissue in the spleen.

The organism was recovered in pure culture from the spleen, liver, splenic abscess and mesenteric glands.

Guinea-pig 5. Received $\frac{1}{4}$ -agar slope of *B. abortus* (strain 830).

This animal gave findings similar to those recorded in the case of Guinea-pig 4, with the exception that the temperature reached 105° F. on the third day after inoculation and that it began to drop to normal about the end of the second week.

Guinea-pig 7. Inoculated with *B. abortus* (strain 895). Similar rise in temperature, reaching 105° F. on the third day and returning to normal on the nineteenth day of the disease.

In both cases (Guinea-pigs 5 and 7) the same post mortem and histological findings were obtained. *B. abortus* was recovered from the kidney in No. 5 and from the bone marrow of No. 7. Cultural examination of the heart blood gave negative results in both cases.

Guinea-pig 8. Inoculated with *B. melitensis* (strain 893) ($\frac{1}{4}$ -slope, 48 hours' culture).

The temperature in this case remained high (105-106° F.) for the first ten days, the pyrexia continuing for three and a half weeks.

Post-mortem findings similar to the above three except that splenic and hepatic congestion was more marked.

In order to measure the relative pathogenicity of the two organisms to guinea-pigs, animals of about equal weight were inoculated intraperitoneally with $\frac{1}{4}$, $\frac{1}{2}$ and 1 slope of *B. melitensis* and with 1, 2, $2\frac{1}{2}$ and 3, etc. slopes of *B. abortus*. It was found that $\frac{3}{4}$ of a slope of *B. melitensis* killed a guinea-pig of 240 grms. in 18 hours. To kill a guinea-pig of the same weight in approximately the same time $4\frac{1}{2}$ slopes of *B. abortus* were required. The amount of growth per slope in both cases was practically equal, thus showing that *B. melitensis* is about six times more virulent than *B. abortus* for the guinea-pig. The method is admittedly somewhat crude, since the number of organisms in the M.L.D. has not been determined, but it gives some idea of their comparative pathogenicity.

The following experiment on goats was then carried out:

Two goats *A* and *B* were examined for previous *melitensis* infection. The blood and urine were examined culturally and the serum tested for the presence of agglutinins for *B. melitensis* and *B. abortus*. All tests proved negative.

Goat A then received one 48 hours agar slope culture of living *B. abortus* (900) intravenously (jugular) on 25. iv. 21.

Goat B received a similar dose of *B. abortus* (895) on the same day.

The blood was examined culturally from time to time, a positive result being obtained 48 hours after the injection (only few bacilli). Later, repeated cultural examination on different occasions gave uniformly negative results. The goats showed very little general reaction. They stood the inoculation well, partook of their food as usual and in fact their general health did not apparently suffer. Urine examinations proved negative throughout.

The antibody response to this inoculation was most marked. Thus the agglutinins in the blood of Goat *A* for *B. abortus* (900) were:

Date	Titre
3. v. 21	1 : 400
10. v. 21	1 : 1600
17. v. 21	1 : 3200
24. v. 21	1 : 6400
4. vi. 21	1 : 6400
11. vi. 21	1 : 12800

Date	Titre
22. vi. 21	1 : 25600
29. vi. 21	1 : 25600
5. vii. 21	1 : 12800
12. vii. 21	1 : 12800
19. vii. 21	1 : 6400

The agglutination titre for *B. melitensis* was somewhat less (1 : 6400–1 : 12800) and least of all for paramelitensis strains (1 : 6400 at most).

Absorption tests carried out with this serum were rather troublesome, as in most cases at least 3 or 4 slopes were required to completely absorb $\frac{1}{2}$ c.c. of 1 : 25 dilution of the serum. However, the same results were obtained with this goat's serum as with rabbits' sera mentioned above (see Tables VI and VII).

Unfortunately the goats available on this occasion were not pregnant and, therefore, the phenomena of abortion, the excretion of bacilli in the milk, and the presence of agglutinins in this latter secretion could not be demonstrated.

CROSS IMMUNISATION.

An attempt was made to find out whether previous immunisation of monkeys with *B. abortus* could ward off a subsequent melitensis infection. The result is of interest and deserves detailed mention:

1. *Macacus rhesus*. Received three doses ($\frac{1}{2}$, $\frac{1}{2}$ and $\frac{1}{2}$ slope of killed *B. abortus*) intravenously at intervals of ten days. The serum finally agglutinated *B. abortus* 1 : 6400 and *B. melitensis* 1 : 3200.

2. *Macacus sinicus*. Not previously immunised, to act as control.

Both monkeys were inoculated on July 1st with $\frac{1}{2}$ -slope of *B. melitensis* intravenously (living culture).

Both stood the infection fairly well for the first 48 hours, and then changes began to appear. The Rhesus (No. 1) continued to take his food and to play as usual, whereas the Sinicus (No. 2 control) became dull, weak, lazy and was inclined to scratch his forehead and to pull some of his crown hair, as if suffering from headache.

At the end of the first week the serum of No. 1 showed a higher titre for *melitensis* (1 : 6400) and *abortus* (1 : 10000) and the blood culture was negative. The second monkey gave agglutination in 1 : 800 and the blood culture was positive (scanty growth).

The accompanying temperature charts show the febrile reaction of *M. sinicus* (No. 2 control) reaching as high as 105.5° F., whereas *M. rhesus* (No. 1) did not show any rise beyond 103.5° F., and this on one occasion only (sixth day).

As regards weight the following table shows that, whereas both monkeys lost weight immediately after the inoculation, the Rhesus monkey soon returned to normal, whereas the control monkey continued to lose weight:

	<i>M. rhesus</i> (No. 1)	<i>M. sinicus</i> (No. 2 control)
Before inoculation	2500 gms.	1950 gms.
3. vii. 21	2480	1910
6. vii. 21	2450	1900
9. vii. 21	2330	1800
20. vii. 21	2500	1700
1. viii. 21	2530	1560

The experiment shows quite clearly that the immunisation of Monkey No. 1 (Rhesus) with *B. abortus*, had been able to protect that monkey against an infecting dose of *B. melitensis*, which in the control non-immunised Monkey No. 2 (Sinicus) produced a quite definite febrile illness.

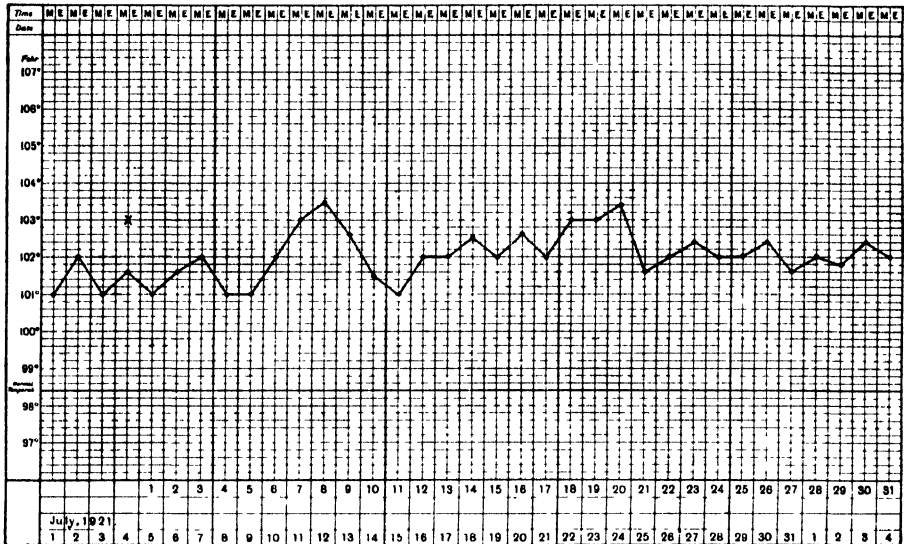


Chart 1. *M. rhesus* ♀ immunised with *B. abortus* and afterwards infected with *B. melitensis*.



Chart 2. *M. sinicus* ♂ control (non-immunised) infected with *B. melitensis*.

PATHOGENICITY TO MAN.

Naturally the question crops up whether or not *B. abortus* being so closely related to *B. melitensis*, is capable of producing an undulant or other form of fever in man. In this connexion I repeat the words of Kennedy (1914): "I think the possibility of melitensis infection of cows in this country should not be lightly thrust aside. I have heard of two cases of undulant fever in people who have never been out of England and it is possible that there are others undiagnosed." I have myself seen cases in Egypt which have never had a chance of ingesting goat's milk and yet suffered from typical melitensis fever as confirmed by laboratory diagnosis.

Meyer and Fleichner (1920) were able to produce in monkeys an undulant-like fever with, in some cases, a fatal result, by means of *B. abortus* (feeding and inoculation).

Cooledge (1916) found anti-abortion bodies in the serum of human beings fed on milk from cows which were suffering from contagious abortion.

There is, however, the fact that undulant fever is unknown in countries where goat's milk is not an important article of food, even though contagious abortion may be widespread. This seems to me more apparent than real. The geographical distribution of undulant fever has been steadily widening since 1886 when it was first known to be a definite clinical entity with a specific organism. Before that time it was often mistaken for a transient fever, for typhoid or for early phthisis, having a very variable symptomatology. The low virulence of *B. abortus* as compared with *B. melitensis* brought out in the experiments on guinea-pigs, would indicate that a larger dose would be required to infect; but, apart from this, there is no reason why *B. abortus* should not produce a febrile condition. In this respect the possibility of the ingestion of large quantities of milk producing a passive immunity to *B. abortus* might require consideration.

Whether the two organisms are one and the same or not and whether the lowered virulence and the different behaviour of *B. abortus* in the absorption tests are produced by passage through cows, I am not ready, at present, to say. It may be that *B. abortus* bears the same relation to *B. melitensis* as cow-pox to small-pox. The fact that cross immunisation of monkeys is successful seems to enhance this supposition, but it is, of course, inadvisable to draw conclusions from a single experiment.

SUMMARY.

1. Morphologically *B. abortus* and *B. melitensis* are identical. The "coccoid" form is not a constant feature and a more satisfactory generic name would be "Brucella."

2. The organisms cannot be differentiated by cultural, bio-chemical, or staining methods, or by the agglutination reaction.

3. From absorption experiments, it would appear that *B. melitensis* is a sub-strain of *B. abortus*.

4. Dose for dose *B. abortus* is much less virulent for the guinea-pig than *B. melitensis*, approximately about 1 : 6.

5. Immunisation of monkeys (one experiment only) with killed suspensions of *B. abortus* protected against subsequent infection with *B. melitensis*.

In conclusion I wish to thank Professor Ledingham for much valuable advice throughout the investigation, and Dr R. St John Brooks for supplying me with cultures from the National Collection.

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Only works to which I have referred in the text of the paper are included in this list.

The Reports of the Royal Commission for the study of Mediterranean Fever (1905-1907) as well as the reports of the Departmental Committee of the Board of Agriculture and Fisheries on Epizootic abortion (1909-1910) contain a great deal of useful information.

THE PERMANENCE OF THE SEROLOGICAL PARATYPHOID B TYPES, WITH OBSERVATIONS ON THE NON-SPECIFICITY OF AGGLUTINATION WITH "ROUGH" VARIANTS.

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(With 1 Figure.)

CONTENTS.

	PAGE
1. Introduction	330
2. "Substrain" variants	331
3. "Rough" variants	335
4. Non-specific agglutination between "rough" alien strains .	335
5. The serological diagnosis of "rough" strains	336
6. The diagnosis of "rough" strains by their growth inhibitions	338
7. The serological paratyphoid B types	339
8. Cross-immunity within the paratyphoid B group	339
9. <i>B. enteritidis</i> Gartner and <i>B. paratyphosus</i> B agglutinations .	340

I. INTRODUCTION.

IN a previous paper (Schütze, 1920), a serological classification of strains giving the Salmonella cultural reactions was described. By segregating those showing agglutinatoriness to one another, two groups were arrived at—the *P. enteritidis* Gärtner and the *B. paratyphosus* B. This latter includes types of diverse origin and pathogenicity, distinguishable in the laboratory by the absorption test; it embraces on the one hand the type labelled "Schottmüller," the well-known virus of paratyphoid B fever, on the other, with perhaps the least agglutinatoriness to it of any in the group, the more recently described "Hirschfeld" type (the *B. paratyphosus* C of that author). Between these extremes, with varying degrees of agglutinatoriness to the "Schottmüller" type, come organisms associated for the most part with food poisoning and animal epidemics, for example, the "Mutton" type and the "Hog Cholera" type. In no case can agglutination alone definitely decide to which type a particular strain belongs; only by absorption can certainty be obtained.

The degree of permanence possessed by serological bacterial types, in particular those demarcated by the finer distinctions of the absorption test, is not known.

Observations made upon the constancy of type manifested by a large number of strains during several years of laboratory cultivation are recorded

here, the species being *B. paratyphosus* B with its numerous absorption types. The results are of importance as indicative of the extent to which prototypes maintain their reliability as such.

2. "SUBSTRAIN" VARIANTS.

Criticism has been levelled at the subdivision of the paratyphoid B group by the absorption test on the ground that by choosing a different member of a serological type as prototype and working with serum derived from it, one arrives at a different arrangement of the strains within the types. But this does not appear to be the case, if notice be taken of the existence of what I have called "substrains." A substrain is one which contains less effective agglutinator antigen than another of the same type. A substrain will agglutinate with and absorb the agglutinins from the serum of a superstrain of the same type more or less badly according to the extent to which it is deficient in effective agglutinator antigen, but the superstrain always agglutinates to titre limit with and absorbs the agglutinins from the serum prepared from a substrain of the same type.

The most striking demonstration of a substrain is afforded by "Piper 1," a culture received from Capt. Fletcher and isolated by him from the urine of a paratyphoid case in 1917. Normal "Schottmüller" organisms were obtained simultaneously from the faeces. Recognised as atypical, the culture was sent to me for identification. On plating, the culture yielded two types of colonies, both giving the Salmonella sugar reactions; while one showed typical morphology, the other was of the kind now called "rough." With this latter, as it was a self agglutinator, little could be done. The former, "Piper 1," was found to agglutinate very poorly with and absorb not at all the Schottmüller serum

Table I.

Serum		Organism agglutinated	Titre						
“Tidy” serum: Unabsorbed		“Tidy”	1 12800						
		“Piper 1”	1 400						
Absorbed with “Piper 1” (1 slope)		“Tidy”	1 12800						
		“Piper 1”							
“Piper 1” serum: Unabsorbed		“Piper 1”	100	200	100	500	1000	3200	Control
		“Tidy”	+++	+++	++	++	+	-	-
Absorbed with “Piper 1” (10 slope)		“Piper 1”	-	-	-	-	-	-	-
	“Tidy”	tr	-	-	-	-	-	-	-
“Tidy”	(10 “)	“Tidy”	+++	+	-	-	-	-	-
	(20 “)	“Tidy”	-	-	-	-	-	-	-
“Tidy”	(10 “)	“Tidy”	+	-	-	-	-	-	-
	(20 “)	“Tidy”	+++	+++	+	-	-	-	-

Absorption took place by adding the amounts of culture indicated to 1 c.c. of serum, diluted in the case of "Tidy" serum to 1₀0 and in the case of "Piper 1" serum to 5₀0, and incubating for 1 hour.

"Tidy" (see Table I). It could not on absorptive or even agglutinary grounds be regarded as of that type. As it conformed no better to any of the other paratyphoid B types, it was decided to carry out the so-called "mirror" test, *i.e.* to prepare a serum from the strain in question and absorb it with the various type strains. The rabbit yielded a serum with titre no higher than $\frac{1}{1600}$ and further inoculation failed to raise it. It will be seen from Table I that this lowness of titre was not due to any inagglutinability on the part of "Piper 1" itself; there was no better agglutination with the typical Schottmüller strain "Tidy." It will be seen too that absorption of the specific agglutinins is performed equally well by both "Piper 1" and "Tidy."

It would seem as if the antigen mosaic were defective in the case of "Piper 1," only a very small portion of that contained in a typical strain like "Tidy" being present or at any rate capable of engaging in the processes of agglutination, absorption and agglutinogenesis. The effective portion has, however, its counterpart in the more complete mosaic of the typical "Schottmüller" strain. "Piper 1" was therefore called a substrain of the "Schottmüller" type.

But further absorptions of "Piper 1" serum with the other paratyphoid B types proved that the classification of "Piper 1" was not so simple, for of the ten absorption types two besides "Schottmüller" were capable of absorbing the specific agglutinins from "Piper 1" serum, *viz.* the "Mutton" and the "Stanley" types.

Here, then, were three paratyphoid B types closely related agglutinatorily yet quite distinct absorptively, all of which completely absorb "Piper 1" serum. "Piper 1" is therefore a substrain to all three equally. The agglutinary antigen in "Piper 1" is apparently so limited in amount that it merely represents that antigen or a portion of that antigen which, in their more complex mosaics, "Schottmüller," "Mutton" and "Stanley" types have in common and by virtue of which they display their agglutinary relationship. "Piper 1" is, as it were, a common denominator of the three types in question.

Five other strains, resembling "Piper 1" in all respects, have been encountered, but none of them with a history indicating that it was isolated as such; one indeed ("Shanks") when received in 1916 was absorptively a normal "Schottmüller," when retested in 1919 it was seen to have degenerated into a substrain similar to "Piper 1." Two others, "Lister" and "Edinburgh," obtained from Prof. Stenhouse Williams and one old laboratory culture, "Wassermann," had no history, but presumably they had not been regarded as coinciding with paratyphoid B "Schottmüller," for they all three had "Supestifer" prefixed to their names. The fifth is described later. What clinical significance is betokened by the fact of an organism proving to be a substrain, it is impossible to say. It may be that some of the cultures isolated from time to time and termed inagglutinable paratyphoids are members of this antigenically very depleted group.

Not all substrains differ from the typical so markedly as these six. Simply

by making single cell cultures from a normal "Schottmüller," it was possible to separate out strains (Tidy A and Tidy B) that were in slight and varying degrees substrains to the original culture (Tidy).

Table II shows a series of absorptions demonstrating incompleteness when substrain acts on superserum and completeness when matters are reversed.

Table II.

Serum	Organism agglutinated	Titre							Control
		200	100	500	1000	2000	5000	12500	
"Tidy" serum:									
Unabsorbed	"Tidy"	+++	+++	+++	+++	+++	++	++	-
Absorbed with "Tidy"	"	-	-	-	-	-	-	-	-
" " "Tidy A"	"	++	++	++	++	tr	-	-	-
" " "Tidy B"	"	++	++	++	++	tr	-	-	-
"Tidy A" serum:									
Unabsorbed	"Tidy A"	+++	+++	+++	+++	++	+	-	-
Absorbed with "Tidy"	"	-	-	-	-	-	-	-	-
" " "Tidy A"	"	-	-	-	-	-	-	-	-
" " "Tidy B"	"	++	++	+	tr	-	-	-	-
"Tidy B" serum:									
Unabsorbed	"Tidy B"	+++	+++	+++	+++	+++	+	-	-
Absorbed with "Tidy"	"	-	-	-	-	-	-	-	-
" " "Tidy A"	"	-	-	-	-	-	-	-	-
" " "Tidy B"	"	-	-	-	-	-	-	-	-

Only the strain "Tidy" with complete antigenic mosaic can absorb from all three sera. The first substrain "Tidy A" can do so from its own and the serum beneath it in the scale, but fails to absorb from its superserum "Tidy," while the second substrain "Tidy B" cannot do so from either of its supersera "Tidy" or "Tidy A." The incompleteness of absorption is not so marked in these cases as it is where "Piper 1" is concerned nor is their antigen so reduced that the heterologous types "Mutton" and "Stanley" contain their counterparts and are thus capable of absorbing their sera. As both "Tidy A" and "Tidy B" can absorb "Piper 1" sera, though the reverse does not occur, the relationship which the strains and types bear to one another may be illustrated as in Fig. 1 (p. 334).

Another instance of prolonged cultivation producing a substrain is afforded by the single cell culture "Tidy A" which, together with the single cell culture "Tidy B," was, as a test of the permanence of their substrain characters, daily subcultured from broth to broth. At the end of about two months "Tidy A" no longer belonged to substrain I but, like the five other cultures already mentioned, to substrain III; it was also found to contain the "rough" variant. "Tidy B" subjected to about half that amount of subculturing had, however, undergone no change.

It has been observed that small changes of position in the scale of antigenic activity, as indicated by the absorption test, often occur. Only twice has a big alteration been recorded, in both cases a descent in the scale; "Shanks" with infrequent agar subculturing during the course of three years and the single cell culture "Tidy A" after 71 broth subculturings had both lost all

effective agglutinatory antigen beyond what is found in members of sub-strain III.

With most variations from the normal, diagnosis is easily accomplished by the mirror test. When a culture has been debased to as low a level as that of substrain III in the diagram, it is not possible to allocate the culture to one particular type; *e.g.* "Piper 1" may equally well be a degraded strain of any one of the three types, "Schottmüller," "Mutton" or "Stanley." Another feature common to substrains is the difficulty experienced in making with them agglutinating sera the titres of which in any way equal those easily arrived at with full normal strains.

After intravenous inoculation with heat-killed saline emulsions (doses of 500 million, 2000 million and 6000 million with six to seven days interval)

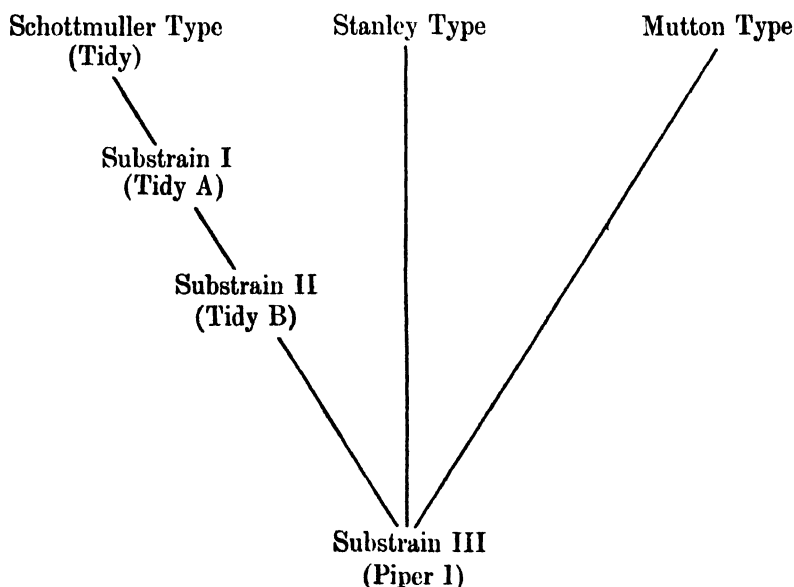


Fig. 1.

rabbits when they receive superstrains yield titres of $\frac{1}{8000}$ to $\frac{1}{12000}$, whereas when inoculated with substrains, they show titres of round about $\frac{1}{1600}$ and further and larger inoculations have little or no effect in raising the potency of the sera. The substrains are thus obviously less effective from an agglutinogenic point of view also.

In the estimation of the agglutinin content of a serum, such markedly substrain cultures as "Piper 1" are likely to make difficulties. In titrating a superstrain serum with a substrain emulsion, for example, one would naturally have to allow for its poor agglutinability, but if it is a serum prepared from a substrain similar to the one being measured, the substrain will register the titre to the full and the employment of any factor arrived at in work done with a superserum would lead one to erroneous results.

When the existence of these substrains is taken into account, the choosing of a different member of the same type as a prototype does not lead to an altered classification.

During the course of this work, it has been seen that even those strains whose serology was registered so long as six years ago, still maintain their places in the same absorptive types. Serologically no alteration except in "altitude" within the type has been recorded after laboratory cultivation extending over that period.

3. "ROUGH" VARIANTS.

There is one other variation that can and does take place during the conservation of cultures, and that is the one recently investigated by Arkwright (1921). The variant has been termed by him a "rough" and it differs from the parent form mainly in the morphology of its colonies, in its stability in saline emulsion, in its appearance in broth culture and in its serological character.

The variation from the normal in the case of the agar grown colonies may be anything from a mere occasional indentation in the edge of an otherwise normal colony to so marked a roughening and flattening of the surface that the growth resembles that of a spore-bearer on potato. In broth culture every stage is seen between a variant that sediments so completely as to leave an absolutely clear supernatant and one that shows but the slightest trace of abnormal precipitation at the bottom of a normally turbid broth culture.

Variations in colonial form and in saline stability do not go hand in hand. The variant giving the roughest colony is not necessarily the most saline unstable and vice versa.

4. NON-SPECIFIC AGGLUTINATION BETWEEN "ROUGH" ALIEN STRAINS.

The other variation already referred to, is a serological one. To a greater or less extent both agglutinary and absorptive relationship to the original culture is lost and, judged on these grounds, the variant could in some cases be regarded as a new type. The degree of alteration in serological character varies independently of the amount of colonial "roughness" and saline stability possessed by the variant. Although little or no agglutination may take place with a rough strain and its homologous smooth serum, the variant is not inagglutinable, it will respond well to a serum that has been prepared from a rough strain. And, what is very remarkable, is that the rough strains possess the power of agglutinating to a considerable extent with the sera of quite alien species when those sera have been made from rough strains. There exists a serological cosmopolitanism among rough cultures. Thus, for example, rough variants of "Gärtner," paratyphoid A and typhoid strains will agglutinate, sometimes to titre limit, with rough sera of the paratyphoid B group, while the smooth prototypes from which they have been derived, remain quite unaffected. Table III gives the titres of several smooth and rough strains for three rough alien sera.

Table III.

			Rough paratyphoid B sera		
Smooth and rough organisms of species alien to the agglutinating sera			"Schottmüller" type	"Hirschfeld" type	"Hog Cholera" type
			Titre = $\frac{1}{32100}$	Titre = $\frac{1}{32100}$	Titre = $\frac{1}{32100}$
Gärtner, "D. H. Bainbridge"	Smooth		$< \frac{1}{100}$	$< \frac{1}{100}$	$< \frac{1}{100}$
"	"	Rough	$\frac{1}{1600}$	$\frac{1}{3200}$	$\frac{1}{6400}$
Paratyphoid A, "S. O."	Smooth		$< \frac{1}{100}$	$< \frac{1}{100}$	$< \frac{1}{100}$
"	"	Rough	$\frac{1}{3200}$	$\frac{1}{3200}$	$\frac{1}{3200}$
Shiga, "550"	Smooth		$< \frac{1}{100}$	$< \frac{1}{100}$	$< \frac{1}{100}$
"	"	Rough	$\frac{1}{1600}$	$\frac{1}{1600}$	$\frac{1}{1600}$
Typhoid, "Howard"	Smooth		$< \frac{1}{100}$	$< \frac{1}{100}$	$< \frac{1}{100}$
"	"Guy"	Rough	$\frac{1}{1600}$	$\frac{1}{400}$	$\frac{1}{800}$

It is seen that while the rough strains disclose an affinity for rough alien sera, the smooth strains fail to do so. That it was not a question of agglutination by serum *per se* whether immune or normal, was demonstrated by controls in which the rough strains remained unaffected. Indeed, for these rough strains serum has in certain concentrations an anti-agglutinator effect and this may be the reason for the frequency with which inhibition zones are met with when agglutinating rough strains.

It would seem then, as if there were genuine affinity between rough strains as such, but the relationship is not closer than that implied by the agglutination test. By absorption even the more closely related of the heterologous rough strains can be differentiated and the homologous ones identified, just as is possible with smooth strains.

5. THE SEROLOGICAL DIAGNOSIS OF "ROUGH" STRAINS.

In Table V are recorded absorptions carried out with a rough "Hog Cholera" serum. "Arkansas," like the strain labelled "Swine Fever," which, similar in every respect, was obtained from the Royal Veterinary College, is an old laboratory culture, and both have gone rough in the course of years of cultivation. To compare the identity of these cultures with more recently isolated and still smooth strains such as the "Hog Cholera XII" of Tenbroeck was impossible with the organisms in their respective states of roughness and smoothness. Table IV indicates what lack of reciprocity there is.

As it is apparently impossible to reconvert a rough into a smooth, Tenbroeck's culture was rendered rough by inoculating from broth to broth; after 7 days at 37° C. the second broth in the series showed signs of abnormal sedimentation and agar plating yielded the rough variant. Absorption of the

rough "Arkansas" serum was then quantitatively carried out with this rough "Hog Cholera" variant as well as with "Arkansas" itself and a rough variant

Table IV.

Serum	Organism agglutinated	Titre									
		$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	Control
"Arkansas" (rough) serum: Unabsorbed	"Arkansas" (rough)	+	+	+	+	+	+	+	+	+	-
"	"Hog Cholera XII" (smooth)	+	+	+	+	+	+	+	+	-	-
Absorbed with "Hog Cholera XII" (smooth)	"Arkansas" (rough)	+	+	+	+	+	+	+	+	+	-

Table V.

Serum	Organism agglutinated	Titre									
		$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	$\frac{1}{100}$	Control
"Arkansas" (rough) serum: Unabsorbed	"Arkansas" (rough)	+	+	+	+	+	+	+	+	+	+
Absorbed with "Arkansas," rough ($\frac{1}{2}$ slope)	"	-	-	-	-	-	-	-	-	-	-
"	" ($\frac{1}{3}$ slope)	tr	-	-	-	-	-	-	-	-	-
"	" ($\frac{1}{3}$ slope)	+	+	+	+	+	+	+	+	+	-
"Hog Cholera XII," rough variant ($\frac{1}{2}$ slope)	"	-	-	-	-	-	-	-	-	-	-
"	" ($\frac{1}{3}$ slope)	+	-	-	-	-	-	-	-	-	-
"	" ($\frac{1}{3}$ slope)	+	+	+	+	+	+	+	+	+	-
"Hirschfeld," rough variant (1 slope)	"	+	+	+	+	+	+	+	+	+	-

of the so closely related "Hirschfeld" (paratyphoid C) type which, indeed, Tenbroeck (1920) considers to be serologically identical with "Hog Cholera."

The rough "Hog Cholera" variant absorbs as well as the homologous

strain and it is therefore justifiable to regard "Arkansas" as a rough variant of the "Hog Cholera" type. The rough "Hirschfeld" variant, on the other hand, discloses a relationship to, but no identity with "Arkansas." And as the mirror test proved that it was not a substrain, a similar close relationship but lack of identity has been demonstrated between the rough variants of the "Hog Cholera" and "Hirschfeld" types, just as had previously been shown to exist between their smooth forms.

Many of the old standard laboratory cultures are found to have become rough during their years of conservation. Though a cultural diagnosis remains possible, their serological characters are obscure unless one takes into account the fact that they are variants from the normal. They are to be investigated, not so much by agglutination, which it has been seen, may be misleading, but by absorption, a test which apparently remains specific. The diagnosis of the rough strain "Arkansas," as here described, indicates in what manner the absorption test is to be performed. Given an unknown "rough" culture, one would prepare from it an agglutinating serum and then proceed to establish which of the type strains (in the form of their rough variants, of course) displayed affinity by completely absorbing the specific agglutinins from the serum.

6. THE DIAGNOSIS OF "ROUGH" STRAINS BY THEIR GROWTH INHIBITIONS.

There is one other method that may help in the typing of such rough strains and that is the investigation of their growth inhibitions. Attention has recently been recalled to this phenomenon in a paper by McLeod and Govenlock (1921). But here the old Eijkman (1904) procedure of direct inoculation of one strain upon another has been employed. By growing an organism in gelatine at 37° C. for 24 hours, cooling the culture to solidification and inoculating the sloped surface with a loopful of broth culture, one can determine what inhibitions to the growth of other organisms have been established. After one or two days at 22° C. if the inoculated bacillus is not inhibited, a roughening shows up along the track of the loop contrasting with the smooth surface of the gelatine and gradually developing into a definite line of growth.

A comparison of the mutual inhibitions and resistances of smooth and rough variants of the "Hirschfeld" and "Hog Cholera" types with those of the two rough strains "Arkansas" and "Swine Fever" gave the following results. Both "Hirschfeld" variants were capable of inhibiting the growth of both "Hog Cholera" variants as well as that of the two strains "Arkansas" and "Swine Fever," whose diagnosis is in question. On the other hand, neither the "Hog Cholera" variants, nor "Arkansas," nor "Swine Fever," though inhibition between themselves was complete, could inhibit either of the "Hirschfeld" variants. In this test also "Arkansas" and "Swine Fever" agree with the "Hog Cholera" rather than the "Hirschfeld" type. How far this method may be trusted to differentiate closely related serological types remains to be seen. Particularly will it be of convenience, if in this respect

substrains behave like the superstrains of their type and there is thus no necessity to prepare a special serum for the unknown strain in order to perform the mirror test. Laboratories would be relieved, too, of the need of preserving sera corresponding to the various types of this very large paratyphoid B group.

7. THE SEROLOGICAL PARATYPHOID B TYPES.

To the nine of these types, described in a previous paper, has subsequently been added a tenth, the "Abortus Equinus" type. This organism, the frequent cause of abortion in mares (Murray, 1919) is a *Salmonella* with marked agglutinary affinity to "Schottmüller" and its allied types, but absorptively quite distinct. The four most commonly encountered types remain: (1) "Schottmüller" (paratyphoid B fever), (2) "Mutton" (food poisoning cases and animal epidemics—it has been recovered from the rabbit, guinea-pig, mouse, calf, duck, parrot, pig, skunk, etc.), (3) "Hirschfeld" (paratyphoid C fever), (4) "Hog Cholera" (associated with the disease of that name); the remaining six have occurred sporadically in man and animals, in food poisonings and continued fevers.

8. CROSS-IMMUNITY WITHIN THE PARATYPHOID B GROUP.

The various types possess to a considerable degree the power of cross-immunisation as witnessed by the following Table VI. The rabbits were all immunised in the same fashion with 24 hours' living broth cultures of the various types—first inoculation 0.01 c.c. subcutaneously, second 0.01 c.c. and third 0.1 c.c. intravenously. After a lapse of 14 days the animals all received varying amounts of a 24 hours' broth culture of Tenbroeck's "Hog Cholera XII," an organism which is so lethal for rabbits that it kills when as few as

Table VI.

Rabbits immunised with living cultures of:	Immunity tested with living "Hog Cholera XII"					
	1 million \times M.L.D.		100,000 \times M.L.D.		10 \times M.L.D.	
	Survivals	Deaths	Survivals	Deaths	Survivals	Deaths
<i>Paratyphosus</i> B, "Schottmüller"	1*	0	0	6	0	3
„ "Hirschfeld"	1	0	2	0	1	0
„ "Mutton"	1	0	1	0	1	0
„ "Reading"	—	—	1	1	1	0
„ "Newport"	1*	0	—	—	—	—
„ "Stanley"	—	—	0	1	—	—
<i>B. enteritidis</i> , "Gärtner"	—	—	—	—	1	0
<i>B. coli</i> , "Escherich"	—	—	—	—	0	1
<i>Streptococcus faecalis</i>	—	—	—	—	0	1
<i>Paratyphosus</i> B, "Hog Cholera"†	—	—	—	—	0	2

* These two survivors were retested after a lapse of a year and still showed immunity to 10 and 100,000 \times M.L.D. respectively.

† Immunisation necessarily carried out with killed vaccine. A third rabbit succumbed to a single M.L.D.

50 to 100 single organisms are injected subcutaneously. The only exceptions to this scheme were the three "Hog Cholera" rabbits, which, because of the virulence of that type, were given the same doses killed by heating to 55° C. for half an hour, and the *Streptococcus* rabbit, which, being an animal immunised for other purposes, had received nine inoculations of killed streptococci (3000 million–15,000 million) followed by two of living streptococci, each 1000 million, all intravenous.

The results confirm Tenbroeck's statement (1918) that while the "Mutton" type (his "Swine Typhus" or animal paratyphoid B) protects against inoculation with "Hog Cholera," the "Schottmüller" type fails to do so. As far as the number of rabbits employed allows of a conclusion, other types besides "Mutton," including *B. enteritidis* Gärtner, also protect, but as has also been shown by Pratt Johnson in work published elsewhere, those rabbits which are immunised with *killed* cultures of the homologous "Hog Cholera" type, remain unprotected. In the reading of such results it must always be borne in mind that an apparently normal rabbit may have survived spontaneous infection by, say, the "Mutton" type and thus have acquired an unsuspected immunity against "Hog Cholera" inoculation, an immunity not only unsuspected but undemonstrable by serum examination, for the "Mutton" agglutination titre may have disappeared by the time the serum is tested. Both "Mutton" type paratyphoid B and *B. enteritidis* Gärtner are frequent invaders of animal houses and it is possible for the results of such immunity work to be confused.

9. *B. ENTERITIDIS* GÄRTNER AND *B. PARATYPHOSUS* B AGGLUTINATIONS.

As statements are made from time to time that these two organisms possess unstable serological characters and that a strain may at one time show those of a normal "Gärtner," and at another those of a normal "Mutton" or some intermediate stage between the two, it may be worth while noting here that not only have the laboratory conserved cultures of these two species constantly maintained their original characters, but in a number of isolations made during the investigation of spontaneously occurring epidemics in the animal house, no organism which could in any way be regarded as intermediate between the two, has been encountered. From animals dying spontaneously or in the course of experimentation (diet deficiencies or tubercle inoculations), some 116 "Gärtner" and 21 "Mutton" strains were isolated—111 of the former were recovered from rats and mice, 14 of the latter from guinea-pigs—and in every case the nature of the organism was unequivocally declared without any indication of a confusing serological cross-relationship.

SUMMARY.

1. Among a large number of strains belonging to the various absorption types of the paratyphoid B group and kept under observation in laboratory culture over a number of years, constancy of type has been demonstrated.

2. Only two alterations in the serological nature of certain cultures have been noted; they were due (a) to the development of so-called "rough" variants, (b) to the degeneration of strains into antigenically less effective "substrains."

Though both are variations within the limits of the absorption type, the serological character of the affected strains may be so obscured that a greater variation than has actually taken place may be ascribed to them, unless the precautions indicated are observed.

3. When "rough" strains are in question, agglutination results are to be mistrusted, for a marked cosmopolitanism in respect of this test has been seen to exist among such alien species as *B. enteritidis* Gärtner, *B. paratyphosus* A and *B. paratyphosus* B.

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STUDIES ON THE EFFECTS OF TUBERCULIN.

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(With 2 Text-figs.)

1. INVESTIGATIONS CONCERNING THE EFFECTS OF TUBERCULIN IN THE HEALTHY ORGANISM.

THE problem of the effects of tuberculin¹ in the organism in many respects is unsolved. To throw some light thereon has been the main object of the experiments here described in this paper.

In his original paper Koch (1912) stated that an injection of 2 c.c. of tuberculin into a healthy guinea-pig to all appearances had no effect. Nevertheless he was of the opinion that tuberculin has toxic effects upon the healthy organism, probably upon the white corpuscles. I shall return to this statement later. Most authors agree that tuberculin has practically no toxic effects upon the normal organism (Behring (1912), Marie and Tiffman (1900), Löwenstein (1908), Ruppel and Rickmann (1910)). Where excessive doses are employed, the toxic effects of the glycerin in the tuberculin will come into consideration.

About the fate of the tuberculin in the organism very little is known. The subject has not frequently been treated in the literature on tuberculin. Slatinéanou and Jonesco-Mihaesti (1908) injected 10 c.c. of tuberculin intravenously into a normal goat. After 12 and 18 days, serum and urine from the goat were injected into tuberculous guinea-pigs. The subsequent rise of temperature in these animals was ascribed to the presence of tuberculin in the goat's blood and urine. Slatinéanou and Danielopol (1908) were, however, never able to demonstrate the presence of tuberculin in the blood of normal guinea-pigs which they had treated with tuberculin, and a similar result was also obtained by Wolff-Eisner (1909). Franceschelli (1913) found that tuberculin was eliminated through the kidneys after 6 hours. By means of the intradermoreaction the tuberculin could be quantitatively recovered in the urine of the animals. Neither by this method nor by that of complement-binding was he able to demonstrate any trace of tuberculin in the blood a few hours after the injection.

To study the fate of tuberculin in the organism more closely I injected it intravenously and intraperitoneally into normal rabbits and guinea-pigs. After a certain interval blood was drawn from the vein or the animal was

¹ "Tuberculin," throughout this paper refers to Koch's old tuberculin.

killed by bleeding. Serum and peritoneal exudate were injected into the skin of tuberculous animals or used for the Pirquet test. As a test-object for this test I have used my own arm, the skin of which is very sensitive to tuberculin. Controls were made with known dilutions of tuberculin. The results were as follows.

A rabbit (weight 5000 g.) was injected intravenously with 2.25 c.c. of tuberculin. If the total amount of blood is estimated to be about 7 per cent. of the bodyweight, a rabbit of 5000 g. will have about 350 g. of blood, and immediately after the injection of 2.25 c.c. of tuberculin it must be supposed to contain about 0.0064 g. of tuberculin per c.c. and 0.2 c.c. of the blood will contain about 0.0013 g., a quantity, which, when injected intradermally into a tuberculous guinea-pig, will give a typical reaction. In fact this proved to be true. 0.2 c.c. of blood drawn immediately after the injection, gave a typical necrosis after injection into the skin of a tuberculous guinea-pig. This experiment, which was repeated several times, shows that tuberculin is circulating in the blood immediately after its injection into the vascular system.

To find out how long tuberculin remains in the circulation, blood was drawn from the vein $\frac{1}{2}$ and $1\frac{1}{2}$ hours after the injection. In serum obtained from this blood tuberculin could not be found by the method described above. Even much greater doses of tuberculin disappear almost immediately from the blood. Thus a young rabbit, weighing 900 g., was injected intravenously with 2 c.c. of tuberculin. Death followed after a few minutes. 12 c.c. of blood, supposed to contain about 0.4 g. of tuberculin, were dried with a fan, and dissolved in as little water as possible. 0.2 c.c. of this liquid, which had a volume of 2 c.c. produced no reaction when injected intradermally into a tuberculous guinea-pig. A small quantity of the solution was smeared into a superficial scarification on my arm in the manner of the Pirquet test, also without reaction, whereas a control with tuberculin diluted 1 to 10 with salt solution gave a very strong positive reaction.

While these experiments clearly show that tuberculin rapidly disappears when injected into the blood, it still remained to be seen whether it disappears as rapidly from other parts of the organism. I therefore injected 1 c.c. of tuberculin subcutaneously into the ear, and 2 c.c. intraperitoneally into a young rabbit (weight 900 g.), which was killed after half an hour. From the ear was obtained a liquid with the appearance and smell of tuberculin. The peritoneal cavity contained about 15 c.c. of a yellowish, cloudy fluid which did not smell of tuberculin. This liquid was steamed down to the volume of 1 c.c. of which 0.2 c.c. was injected intracutaneously into a tuberculous guinea-pig. The effect after 2 days was a superficial necrosis about the size of the half of a sixpence. The control (tuberculous guinea-pig) succumbed after a dose of 0.05 c.c. of pure tuberculin. These injections have been repeated several times both with tuberculous and non-tuberculous animals. In all cases the disappearance of the tuberculin from the peritoneal cavity was rapid and complete.

The foregoing experiments show that tuberculin rapidly disappears when injected intravenously or intraperitoneally into the organism, but they give no information about its fate. Two possibilities may come into consideration. As was shown by Franceschelli (1913) the total quantity of injected tuberculin cannot be found in the urine till after the lapse of several hours. In the meantime the substance must be stored outside the vascular system, or it must be present in the blood in a combination which does not give the tuberculin reaction. Now the question is, has the blood *in vitro* or *in vivo* any power of binding tuberculin?

Experiments which were carried out to solve this question gave a definitely negative result.

By mixing and incubating extract of fresh ox-liver in salt solution with tuberculin, I found no evidence that any of it was fixed, so I proceeded to try if the living cells themselves would have this power.

Guillain and Laroche (1910) found that tuberculin was bound by brain-substance. But according to these authors the mixture of brain and tuberculin was even more toxic than tuberculin alone. By mixing fresh paste of ox-liver and -brain with tuberculin, evidence was found that the tuberculin was bound by the living cells. By boiling it could be recovered from the new combination.

Living cells therefore may fairly be supposed to possess a faculty of binding tuberculin *in vitro*. The possibility remains that this reaction also takes place *in vivo*.

In order to trace the tuberculin in the organism I have injected tuberculin into normal guinea-pigs and rabbits. According to Franceschelli, tuberculin leaves the body through the kidneys and may be recognised in the urine some hours after the injection. This statement I have been able to confirm: 2 guinea-pigs (weight 450 g.) were injected with 1 c.c. of tuberculin. The 24 hours' urine was collected and used for the Pirquet reaction, which was faint, but typical. (Urine from normal guinea-pigs gave no reaction.) A similar reaction was also obtained with urine from the following 48 hours. In both cases the urine was concentrated by steaming and afterwards treated with an equal volume of pure alcohol. The precipitate which formed, was washed with alcohol and dissolved in distilled water. The test was made with this solution. Tuberculin rapidly disappears from the blood, and as it cannot be recovered from the urine until at least 3 hours have elapsed from the time of injection, I killed some animals at this time to find out if the tuberculin can be demonstrated in any of the tissues. The rabbits (weight 900 to 2500 g.) were injected intravenously with 3 c.c. of tuberculin, and the guinea-pigs (weight 300 to 450 g.) received 2 c.c. intraperitoneally.

In the blood I was never able to find tuberculin. The examination of lungs, kidneys, spleen and the contents of the bowels gave no better results. In order to detect even traces of the substance the organs were ground and boiled with water. The filtered decoction was steamed down and pure alcohol was added. The precipitate was dissolved in water and injected into tuberculous guinea-

pigs. Extracts from the liver, which had been prepared in this way, in three cases gave a slight necrosis after injection. In eight cases I got a distinct, though small necrosis after injection of extract from muscles and bones, and in three cases when extracts were prepared from muscles and bones separately, I found that whereas the extract from bones gave a distinct reaction, the extract of muscles had no such effect. By these injections I have, however, never been able to produce as strong a reaction as that effected by pure tuberculin. Control-injections into normal animals were always negative.

It must therefore be concluded that the reaction which takes place *in vitro* by the binding of tuberculin in living tissue to a certain extent may take place also *in vivo*, and that the binding here is mainly effected by the bones. The circumstance that tuberculin cannot be quantitatively recovered in the organism after injection, possibly means that the tuberculin now forms a new combination, which we cannot detect by our methods. This may also explain why we have not been able to find tuberculin in the tuberculous organism. Many attempts have been made to isolate tuberculin from affected organs, but always in vain. My experiments in this respect have been equally without result. Recently I have tried to isolate tuberculin from the bones of tuberculous animals and men. From lack of material I have not, however, been able to complete these experiments.—Not until we obtain certain knowledge regarding the fate of tuberculin in the organism shall we be able to determine, whether it is a true product of the tubercle bacillus or an artefact, formed by the decomposition of the real toxin.

2. EXPERIMENTS WITH COMPLEMENT-BINDING.

The evidence of a certain immunity in tuberculosis is not supported by facts which can give us any definite idea of its exact nature. Whereas in diphtheria and tetanus the immunity can be explained by the presence of substances which have the faculty of neutralising the toxins of these diseases, *in vitro* and *in vivo*, in tuberculosis we have not been able to demonstrate a similar antitoxin. As a means of solving this question, much attention has been paid to the complement-binding antibodies, which, however, as far as we know, play no great part in the immunology of tuberculosis. Although it has been proved that in many cases of tuberculosis in men and animals the presence of complement-binding antibodies may be demonstrated, yet where we most expect to find them, in the blood of individuals which have recovered from their disease, very often no trace of them can be found (*vide* Löwenstein and others).

As previously mentioned, the method of complement-binding has not given very important results in the immunology of tuberculosis. In spite of much effort we still know very little about the relation between the tuberculous antigen and its antibodies. As will be described below I have tried to throw some new light on these questions, using for the purpose a somewhat modified version of the technique, commonly applied in studies of complement-binding.

The object of my experiments has not been to determine the quantity of complement-binding antibodies in tuberculous sera, but to investigate the nature of the alexin in these sera, and the nature of its combinations with antigen and antibodies.

The complement used in my experiments has mostly been complement from human sera. In some cases I have used complement from guinea-pigs and rabbits. Before proceeding to the main experiment the quantity of complement in the sera was always determined by titration. This was carried out by adding active serum in varying quantities to tubes containing equal volumes of washed corpuscles and inactivated rabbit's amboceptor, which was prepared by injecting a rabbit intravenously with sheep's erythrocytes. In all experiments the amboceptor was used in doses which had the power of dissolving twice the quantity of erythrocytes that was used. During the months in which the experiments were made the haemolytic power of the amboceptor remained practically unaltered. It was stored undiluted in the refrigerator. Because of the small quantities of human serum which were at my disposal, it was expedient to use a very thin emulsion of erythrocytes. In all experiments I therefore used a 0.5 per cent. emulsion. The serum was diluted 1 to 10 in all experiments. (Rabbit's serum was diluted 1 to 4.)

In order to determine the complement-quantity of the sera, 0.5 c.c. of a 0.5 per cent. erythrocyte-emulsion and 0.003 c.c. of amboceptor from a rabbit treated intravenously with sheep's erythrocytes, were filled into 10 tubes. To all tubes was added the unheated serum, which was diluted with salt solution, in quantities varying from 0.08 to 0.28 c.c. Finally the contents of the tubes were brought to the same volume by means of salt solution, and the tubes were incubated for $\frac{3}{4}$ of an hour. The smallest quantity of diluted serum, which was sufficient to bring about a complete haemolysis I have called the titre (T) of complement. If the haemolysis was complete in all tubes, or if it was not complete in any of them, the titration had to be repeated with smaller or greater doses of serum.

As the emulsion of erythrocytes is not quite constant from day to day, the titre found by this method is no absolute measure of the quantity of complement contained in the sera. Nevertheless we obtain a relative measure of the contents of complement in sera which are investigated at the same time. The difference between the absolute and the relative values of the titres can, however, not be great. This will be seen from the titres of sera examined on different occasions. Of 349 sera in which the complement was examined, 83 per cent. had a titre between 0.10 and 0.20. 25 per cent. had a titre of 0.12. A small number of sera, having a titre above 0.30, were not used for further experiments. As the blood-samples were always kept under the same conditions, and examined 2 to 24 hours after the drawing of the blood, the diagram (Fig. 1) will give us a good idea of the contents of complement in human sera. The sera belonged to all sorts of persons, old and young (not infants), sound or suffering from various maladies.

As will be seen from Fig. 1, the amount of complement in the sera was found to be constant within certain limits. No relation could be found between the state of health of the donor of the blood and the quantity of complement in his serum.

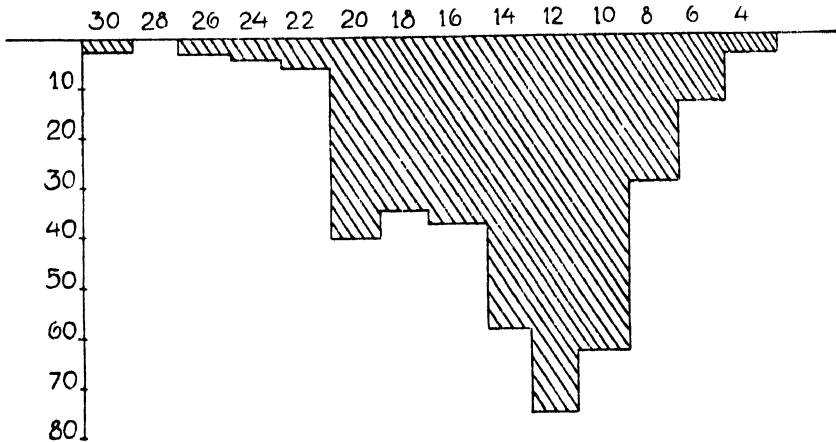


FIG. 1. Diagram showing the complement content of the sera examined.
(Numbers along the top indicate the complement-titre, numbers along the side the numbers of sera examined.)

After the quantitative determination of the complement the main experiment was carried out, the object of which was to compare the combination or binding, which was effected between the tuberculous antigen, alexin and tuberculous amboceptor in the different sera. One experiment, accordingly, always gave me a comparison between two different sera.

Before the experiment eight tubes are arranged in two rows of four (see Fig. 2). All of the tubes receive 0.25 c.c. of a 10 per cent. solution of tuberculin in 0.85 per cent. salt solution. The two sera A and B are filled into the tubes, into the upper row of tubes active serum A and inactivated serum B, into the lower row inactivated serum A and active serum B. The tubes marked I contain exactly the quantity of complement which is necessary to dissolve 1 c.c. 0.5 per cent. emulsion of sheep's corpuscles. But whereas the complement in the upper row of tubes is represented by the complement of serum A, the complement in the lower row belongs to the serum B. The tubes, as will be seen, in all other respects have the same contents. The tubes marked II contain one and a half, the tubes marked III twice, and the tubes marked IV thrice as much complement as the tubes marked I. Finally equal volumes in all tubes are obtained by adding 0.85 per cent. salt solution. Now the tubes are placed in the incubator for $\frac{3}{4}$ of an hour in order to effect the binding of the complement by means of tuberculous antigen and amboceptor. After removal from the incubator 1 c.c. of erythrocyte-emulsion, with twice as much rabbit's amboceptor as is required to dissolve it, is added to all tubes, which are again

put into the incubator for $1\frac{1}{2}$ hours. Now the tubes are examined and the result of the haemolysis is noted.

To exclude errors arising from incomplete inactivation of the sera, controls were often made with the inactivated serum, amboceptor and corpuscles. The inactivation was always complete.

As the tubes in the two rows in couples contain exactly the same amount of antigen, amboceptor, complement and serum, the haemolysis in the two rows

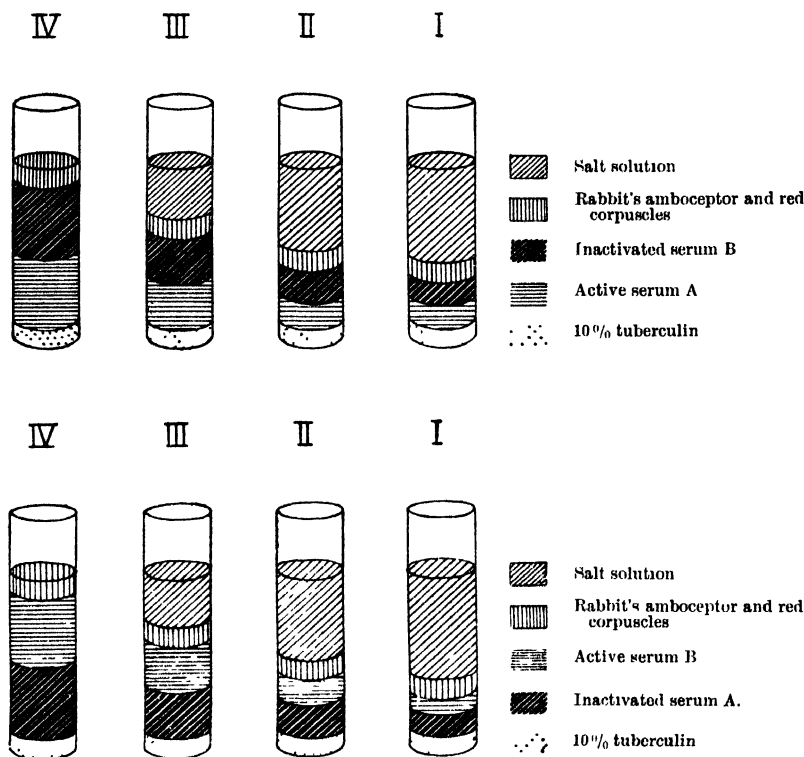


Fig. 2.

must be supposed to be parallel. This is, however, as will be seen from my experiments, not the case.

In my tables XXX means total, and XXO partial haemolysis. XOO indicates traces of haemolysis and OOO no haemolysis.

As the contents of the tubes only differ in regard to the quantities of sera, in my tables I have only written down the complement-titre (T) of the sera, and the results of the haemolysis. The tubes marked II always contain one and a half, the tubes marked III twice and the tubes marked IV thrice as much complement as the tubes marked I.

From the following experiments it will be seen that the haemolysis in the two rows is not parallel although the amounts of complement in the two rows are equal. Similar results which were obtained through numerous experiments, show us that some complements will be more readily fixed than others.

Expt. 1. (17. x. 1918.)

Abbreviations: S = serum. R = rabbit. M = man. G = guinea-pig. T = titre. H = haemolysis.
 Tu = tube. U.r. = upper row of tubes. L.r. = lower row of tubes. A = active. Ina = inactivated.

S. R. 3. Tuberculosis. T 0-14					
S. 23. M. Lues T 0-14					
U.r.	Tu.	4.	3.	2.	1.
					0-14 c.c. A. S. R. 3.
					0-14 c.c. Ina. S. 23.
	H.	000	000	000	000
L.r.					0-14 c.c. Ina. S. R. 3.
					0-14 c.c. A. S. 23.
	H.	XXX	XXX	XXX	XXX

Expt. 2. (17. x. 1918.)

S. R. 4. Tuberculosis. T 0-10					
S. 23. M. T 0-14					
U.r.	Tu.	4.	3.	2.	1.
					0-10 c.c. A. S. R. 4.
					0-14 c.c. Ina. S. 23.
	H.	X00	000	000	000
L.r.					0-10 c.c. Ina. S. R. 4.
					0-14 c.c. A. S. 23.
	H.	XXX	XXX	XXX	XXX

This phenomenon, in my opinion, can only be interpreted in one way. What is called complement or alexin is not the identical substance in all sera, thus the complement in rabbit's serum differs constitutionally from that in human serum.

The view that there are a plurality of complements was first advanced by Ehrlich and Morgenroth (*vide* Ehrlich, 1900), who demonstrated several facts which strongly supported it. They stated that "experimental results positively show a multiplicity of complements in normal serum." This hypothesis has, however, been vehemently opposed by many authors, especially by Buchner and Bordet (*vide* Bordet, 1920), who maintain the existence of a single complement. As will be seen, my observations are in full accordance with Ehrlich's view.

The difference which I could demonstrate between the binding of rabbit's complement and human complement can, as the following experiment will show, also be demonstrated between other heterologous sera, for instance, between the complement of rabbit and guinea-pig.

Expt. 3. (16. xi. 1918.)

S. R. 1. Tuberculosis. T 0-10					
S. G. Normal. T 0-08					
U.r.	Tu.	4.	3.	2.	1.
					0-10 c.c. A. S. R. 1.
					0-08 c.c. Ina. S. G.
	H.	X00	000	000	000
L.r.					0-10 c.c. Ina. S. R. 1.
					0-08 c.c. A. S. G.
	H.	XXX	XXX	XXX	XXX

The complement of 10 guinea-pigs, compared with the complement of 13 human sera in four cases showed some difference.

As will be seen from the experiments which are mentioned above, the comparisons in all cases have been made between heterologous sera. Now the question is, can a similar difference be demonstrated also between homologous sera? The following experiment answers the question affirmatively.

Expt. 9. (29. xi. 1918.)					
	S. 75. M.			T 0.14	
	S. 24. M.			T 0.16	
U.r.	Tu.	4.	3.	2.	1.
					0.14 c.c. A. S. 75.
					0.16 c.c. Ina. S. 24.
	H.	000	000	000	000
L.r.					0.14 c.c. Ina. S. 75.
					0.16 c.c. A. S. 24.
	H.	XXX	XXX	XXO	000

The difference which I have been able to demonstrate between the complements may be ascribed theoretically to the circumstance that one complement is able to make combinations which other complements cannot. These combinations may arise either between specific tuberculous components of the antigen (tuberculin) and the corresponding amboceptors, or between non-specific components and corresponding "accidental" amboceptors in the sera.

In the case of human sera the existence of tuberculous amboceptors is very difficult to exclude, because of the frequent and often unobserved tubercular infections in man. Very often human sera must be supposed to contain both tuberculous and other amboceptors, which are able to unite with non-specific components of tuberculin. In the case of laboratory animals, however, we know to a certain degree the factors with which we have to deal.

Below I will give the results obtained in experiments with homologous sera from normal and tuberculous laboratory animals.

On examining the sera of eight normal rabbits with the technique which I have described, no difference could be found between the complements. But the complements of these eight sera and five other sera, also from normal rabbits, in most cases showed a marked difference from the complements of sera from four tuberculous rabbits.

Expt. 10. (26. x. 1918.)					
	S. R. Tuberculosis.			T 0.14	
	S. R. 20. Normal.			T 0.14	
U.r.	Tu.	4.	3.	2.	1.
					0.14 c.c. A. S. R. Tuberculosis.
					0.14 c.c. Ina. S. R. 20.
	H.	XOO	OOO	OOO	OOO
L.r.					0.14 c.c. Ina. S. R. Tuberculosis.
					0.14 c.c. A. S. R. 20.
	H.	XXX	XXO	OOO	OOO

In most cases where a difference could be demonstrated the tuberculous complement was more completely bound than the normal complement. In four cases no difference could be shown between the two complements.

In one experiment a comparison was made between the complements of a tuberculous and a normal guinea-pig, but no difference could be demonstrated.

The experiments with human sera may be divided into two groups, (1) those with complement derived from persons whose relation to tuberculosis was unknown, (2) those with complement derived from tuberculous persons.

(1) *Experiments with complements from persons who have not suffered from tuberculosis or whose relation to previous tubercular infection is unknown.*

Of this category 182 sera were examined. Twelve of these gave a different reaction to the others.

(2) *Experiments with complements from persons suffering or having previously suffered from tuberculosis.*

Thirty-five sera from tuberculous persons were examined. They were compared with 296 sera from other persons, the majority of which were the sera mentioned under (1).

These experiments show (a) that the complements of different sera not infrequently show differences; the complement of a guinea-pig is not identical with that of a rabbit or of a man; (b) that differences also may be found between complements derived from individuals of one species; (c) that probably the complement of a tuberculous individual differs from that of the healthy subject. Of 182 complements belonging to persons who had not suffered from tuberculosis, or who were not known to have suffered from tuberculosis, only 12 differed from the others. Thirty-five tuberculous sera showed a distinct difference when compared with 207 sera from non-tuberculous individuals, whereas no difference could be demonstrated in 86 cases. In some cases the haemolysis was more complete in the tubes containing tuberculous complement, in other cases it was more complete in the tubes with non-tuberculous complement. As will be seen from the experiments, however, in many cases a striking conformity could be observed and the bindings of tuberculous complements in many cases were absolutely parallel.

I am fully aware of the circumstance that what I have called non-tuberculous complements very often belong to individuals who must be regarded as infected with tuberculosis. This circumstance may, perhaps, account for the cases where a difference between tuberculous and other complements could not be demonstrated. My object has only been to demonstrate a difference between complements of persons who have been subjected to grave tuberculous infections and complements of persons who have not been subjected to tuberculous infections, or only to infections which may be regarded as inconsiderable.

Against the technique which I have used in this work certain objections can be made.

Moro (1907) stated that the titration of human complement is not exact

when performed with the technique which I have described above. In some cases a better result may undoubtedly be obtained by Moro's method, but even if the titration is carried out in the way which is described by him, we cannot be certain of finding the exact amount of complement contained in the sera. In most cases I believe that the ordinary technique will serve its purpose as well as any other. This opinion is based upon the following facts.

1. The amounts of complement which I have found in human sera are on the whole the same as were found by Moro.

2. A great many of my sera, after titration, were mixed with amboceptors from other sera and the complement was titrated repeatedly. (The main experiment is really a titration of the complement.) If the first titration had given a wrong result owing to a lack of human amboceptor, later titrations with addition of different amboceptors never could give the same result in a series of experiments. This was, however, very often the case.

3. In the titration of complement from rabbits and guinea-pigs Moro has no objections against the ordinary technique. These complements, however, showed the same differences as human complements.

As was observed by Fridemann (1910) and others, a normal serum may give a positive Wassermann reaction, whereas this reaction disappears after inactivation. Fridemann has proved that the phenomenon depends upon the globulin-content of the serum, but he also states that it is of no practical importance where sera from men and guinea-pigs are concerned. The difference which I have found between the complements accordingly cannot be explained as an analogy to this phenomenon. And in no case can it explain the difference existing between tuberculous and non-tuberculous complement. In some experiments I have made a control experiment which fully proves that my results have nothing to do either with a deficient method of titration or with the phenomenon observed by Fridemann. One of these control experiments will be related below:

	S. R. Normal,			T. O. 14.	
	S. G. Tuberculosis			T. O. 20.	
U.r.	Tu.	4.	3	2.	1.
					0.20 c.c. S. G. A.
					0.14 c.c. S. R. Ina.
	H.	000	000	000	000
L.r.					
					0.20 c.c. S. G. Ina.
					0.14 c.c. S. R. A.
	H.	XXX	XXX	X00	000

The control experiment was performed without the addition of tuberculin, which was used in the main experiment. Whereas the haemolysis in the latter was as related above, in the control the haemolysis was complete in all tubes. This experiment shows beyond a doubt that the complement-binding by the combination between tuberculin and corresponding amboceptors has occasioned the different results of the haemolysis in the two rows of tubes.

3. EXPERIMENTS WITH PHAGOCYTOSIS.

Summing up the results obtained by innumerable workers with the general and known methods of immunological investigation, one has to admit that the riddle of immunity in tuberculosis is still unsolved, and, bearing this in mind, attempts to solve the problem must necessarily be based upon new methods. I have therefore adopted the methods of Wright for the examination of leucocytes, hoping in them to find a finer indicator of the effects of the tuberculous agents than is represented by the whole organism of men and animals.

The object of my work has been to determine whether tuberculin, the substance which is supposed to be the toxin of the tubercle bacillus, has any toxic effects upon the leucocytes, and whether it affects the leucocytes from a healthy organism in the same way as it affects those from an organism suffering from tuberculosis.

One of the vital functions of the leucocytes is their motility. Does tuberculin affect this motility? In order to study this question I have used the method of the emigration or chemotactic tubes of Wright (1915).

The emigration tube was first filled to midway point with blood from a puncture in the finger and tuberculin added by superimposing it upon the unclotted blood.

When undiluted tuberculin was superimposed upon my blood no emigration of the leucocytes into the white clot took place. This inhibitory effect of the tuberculin on the wandering of leucocytes may be thought to be caused by osmotic conditions in the fluids above the red clot. I found that a 0.5 per cent. salt solution has a lower, and a 1.5 per cent. salt solution a higher molecular tension than tuberculin. As both these concentrations, however, when superimposed upon the blood, effected a marked emigration of leucocytes into the white clot it is obvious that molecular conditions cannot be the cause of the inhibitory effects of tuberculin. As we know, tuberculin consists of glycerin-broth and a decoction of bacilli, and it is therefore possible that the glycerin or the broth or both of them may inhibit the emigration. A 10 per cent. solution of glycerin in broth was steamed down to the consistence of tuberculin, and superimposed upon the blood. This fluid inhibited the emigration of the leucocytes to some extent, but when diluted 1 to 5 with salt solution emigration was abundant. Tuberculin, on the other hand, even when diluted 1 to 5 and 1 to 25 with salt solution, totally inhibited the emigration, whereas a dilution 1 to 50 had no such effect. These experiments clearly show that tuberculin has a distinct and characteristic inhibitory effect upon the motility of leucocytes. The white corpuscles are similarly affected also by tetano-toxin and diphtheria-toxin. Vaillard (1891) demonstrated that tetano-toxin has a negative chemotactic influence upon leucocytes *in vivo*. It is easily demonstrated *in vitro* both for tetano-toxin and diphtheria-toxin. If tetano-toxin, undiluted or diluted 1 to 5, or diphtheria-toxin, undiluted or diluted 1 to 25,

is superimposed upon blood in the way described, no emigration of leucocytes will take place. This toxic effect can, however, be neutralised by antitoxins. Undiluted horse-serum has an inhibitory effect upon the motility of human leucocytes, but when it is diluted and added to toxin, a great number of them will wander into the white clot, if the mixture is superimposed upon blood. The leucocytes in this way give us an indicator of the action of toxin and antitoxin. It may therefore be thought that similar phenomena may be demonstrated also under different circumstances, for instance, where the tuberculous toxin comes into contact with the unknown tuberculous antitoxin. In order to test this hypothesis I incubated mixtures of tuberculin with serum from persons suffering from tuberculosis, and from persons who have conquered the infection, and whose organism accordingly may be supposed to contain antitoxin. The inhibitory effect of tuberculin on the motility of the leucocytes could, however, in no case be neutralised.

The results obtained with the emigration tubes clearly show that tuberculin has an effect upon white blood corpuscles, and that it cannot be regarded as an indifferent substance. In my experiments I have mostly used my own blood as test object, and because I give a positive Pirquet reaction, my blood may be regarded as tuberculous blood, and the effect of tuberculin upon my leucocytes therefore be explained as the effect upon tuberculous cells. This objection is, however, of no value, because leucocytes from normal rabbits and guinea-pigs are affected in quite the same way by tuberculin.

The emigration tubes cannot give us a quantitative measure of the effects of tuberculin upon white corpuscles. In order to obtain this I used the opsonic technique of Wright (1912).

For my purpose I used blood from my finger, and, having washed and centrifuged the corpuscles in the ordinary way they were divided into two equal parts. One of these parts was mixed with tuberculin, and the other with 0.85 per cent. salt solution. Then the tubes were incubated for one hour at 37° C. After this time the tubes were centrifuged and the corpuscles washed three times with salt solution. Samples from both tubes were mixed with the same emulsion of staphylococci and the same serum according to the ordinary technique. The cytophagic index was determined by counting the number of staphylococci which were enclosed in at least 50 leucocytes. By this method one is able to obtain a quantitative measure of the effect of tuberculin upon another of the vital functions of the leucocytes, the phagocytosis.

These experiments showed that tuberculin had a distinct inhibitory influence upon the phagocytosis. Controls proved that this effect was not caused by the contents of glycerine and salts of the tuberculin. The same effect was demonstrated in the tuberculin which had been purified in the way described by Koch (1912, vol. I, p. 674).

The picture which we obtain when staining after having treated the leucocytes with toxin is quite different from the normal. Whereas the latter shows us the leucocytes beautifully stained and enclosing the staphylococci,

in the former the phagocytosis, if present, is decidedly smaller, and the leucocytes are badly stained with indistinct differentiation between protoplasm and nucleus.

In order to evade those constituents of tuberculin which come from the glycerin broth, I have cultivated tubercle bacilli on a solid medium, and afterwards boiled them in sterile, distilled water. After filtering the decoction it was steamed down to dryness at a temperature of 56°C ., and dissolved in 0.85 per cent. salt solution. With this solution, which caused no trace of haemolysis, I have treated the leucocytes, using the same technique as before. Whereas white blood corpuscles which were kept for 2 hours in the incubator at 37°C ., and afterwards mixed with staphylococci and serum, on an average contained nine staphylococci (50 leucocytes and 450 staphylococci counted), those corpuscles which were treated with decoction of tubercle bacilli instead of salt solution, were highly degenerated and contained no staphylococci at all.

These experiments leave no doubt as to the toxic effect of tuberculin on leucocytes and phagocytosis. But about the nature of this effect we know very little. On the one hand it is possible that tuberculin has a similar effect upon the cells as other bacterial toxins (*i.e.* diphtheria toxin), on the other hand there are many who hold that the effects of tuberculin upon the organism must be ascribed to anaphylaxis.

As has been demonstrated by Gengou (1898), diphtheria- and tetano-toxin have no effect upon monocellular organisms, and Bordet (1896) found that a contact of 3 to 4 hours with diphtheria-toxin did not diminish the phagocytic power of leucocytes. My experiments serve but to confirm the results of Bordet.

Working error: In my opsonic work I have estimated my working error at 10 per cent. On counting a great number of control films the results never varied more than 10 per cent. My technique has been very time-consuming, a circumstance which has greatly hampered the taking of controls. On counting these I find that they represent about 15 per cent. of the total number of experiments. In fact the experiments which were made to illuminate the same question control themselves.

As previously mentioned, tuberculin cannot be regarded as a genuine toxin like, for instance, diphtheria-toxin. Now the question is, can the toxic influence of tuberculin upon phagocytosis be ascribed to anaphylaxis?

What we know about the nature of anaphylaxis is that it will manifest itself in a number of quite characteristic clinical symptoms from the nervous, cutaneous, respiratory, vascular, alimentary and the genito-urinary systems, in short from all parts of the body. We know, furthermore, that these symptoms can be reproduced in isolated organs, as for instance in the isolated heart or uterus, and that the state of hypersensitiveness is due to an altered condition of the blood. As an explanation of these phenomena it is supposed by many that the noxious agent which is the cause of the anaphylactic shock has its point of attack in the nervous system (Besredka, 1911). Sufficient experiments have not been carried out to determine whether the anaphylactic

reaction is a universal reaction or not. This possibility must, however, be taken into consideration. In fact some of the manifestations of hypersensitiveness can more easily be explained as a cellular reaction, caused by the influence of the noxious agent upon the so-called sessile receptors in the cells. As mentioned, anaphylactic symptoms can be reproduced in isolated organs. Arnoldi and Leschke (1920) demonstrated anaphylactic reactions in organs which have been completely freed from all traces of blood. The local reaction which is seen as the result of an injection of serum into the skin of animals previously treated with the same serum, although generally accepted as a symptom of anaphylaxis, has no resemblance to the anaphylactic shock, is not necessarily accompanied by symptoms of general hypersensitiveness, and can more easily be explained as a local cellular reaction than as the local result of the influence of the noxious agent upon the nervous system. An experiment which I have carried out points to the conclusion that the reaction may be quite independent of the nervous system. Six to seven days after therapeutic injections of horse-serum, patients were injected with a mixture of horse-serum and novocain-adrenalin which effected a complete anaesthesia. The local reaction was always typical and of the same dimensions as the controls.—Summing up what is really known about anaphylaxis one must therefore admit the possibility of a cellular hypersensitiveness, which may manifest itself with a cellular anaphylactic shock.

In order to investigate this question and in continuance of the experiments described above, I have attempted to produce anaphylaxis in cells from sensitised organisms. As I wanted to work with isolated cells which were capable of living outside of the organism, I have used leucocytes as my test-object, the vital functions of which may be partially controlled by the technique which I have described.

Numerous experiments demonstrated that:

(a) In experimental anaphylaxis the cytophagic index was unaltered before and after the shock, in tuberculous guinea-pigs before and after tuberculin-injections.

(b) The anaphyla-toxin (Friedberger, 1917) does not always affect the cytophagic power of the leucocytes, although in some cases of serum-disease an inhibitory effect of horse-serum upon leucocytes was demonstrable.

If we mix tuberculin with serum from a tuberculous or a normal animal, afterwards exposing leucocytes to the effects of this mixture, we will find that the *noxious influence* of the tuberculin upon the phagocytosis *has been partly neutralised* by the serum. This is demonstrated by the following experiment.

Blood from a normal guinea-pig was treated for $1\frac{1}{2}$ hours at 37° C. with:

(a) Salt solution. Cytophagic index, 5.8. (289 staphylococci and 50 leucocytes counted.)

(b) Tuberculin, diluted 1 to 10 with salt solution. Cytophagic index, 0.98. (49 staphylococci and 50 leucocytes counted.)

(c) Tuberculin, diluted with one volume of serum from a tuberculous

guinea-pig and eight volumes of salt solution. Cytophagic index, 2.66. (133 staphylococci and 50 leucocytes counted.)

(d) Tuberculin, diluted with one volume of serum from a normal guinea-pig, and eight volumes of salt solution. Cytophagic index, 5.18. (258 staphylococci and 50 leucocytes counted.)

The protective power of the serum against the noxious influence of the tuberculin upon the phagocytosis is not removed by the destruction of the complement. Serum from normal animals practically always had a stronger protective power than serum from tuberculous animals. It was, however, not possible to ascertain whether the tuberculous leucocytes were more sensitive to the toxic effects of tuberculin than the normal ones. This is undoubtedly due to a deficiency in the technique. In many cases the films will contain leucocytes which are so altered by the tuberculin that they are unrecognisable. This naturally causes grave errors in the results, and I have therefore not taken the trouble to continue my attempts to solve the question in this way. The toxic influence of tuberculin upon leucocytes can, however, be demonstrated directly in another way by means of the paraffin cells of Wright (1918).

Into one of these cells, with living leucocytes, we put a drop of tuberculin, diluted 1 to 20 or 1 to 100 and neutralised with sodium hydrate. Into a control cell we put a drop of salt solution and place the slide in the moist chamber at 37° C. for 1½ hours. The slide is again withdrawn and the tuberculin is washed off with salt solution. This done, we fix with corrosive sublimate and stain with methylene blue. In the cells where the tuberculin has been a number of the leucocytes have disappeared, and a great number of the remaining leucocytes will present a picture of degeneration. The protoplasm is shrunken and the differentiation between protoplasm and nucleus is indistinct. In some places we find badly stained heaps, which must be regarded as the remnants of degenerated leucocytes.

These methods will, however, not give us a quantitative means of measuring the effect of tuberculin upon normal and tuberculous leucocytes. This may be obtained in another way, which I will describe below.

An injection of pure tuberculin into the peritoneal cavity of a guinea-pig will produce a rich exudation of leucocytes into the peritoneal fluid. If injections are made at the same time into tuberculous and normal guinea-pigs, the exudates will be seen to differ in several respects. Whereas the exudate from the normal guinea-pig will coagulate slowly, the exudate from the tuberculous animal will coagulate within a few minutes. Also the leucocytes of the two exudates are different. This difference can be demonstrated by vital staining. The exudates are centrifuged and washed with salt solution. The leucocytes are mixed with a drop of Manson's stain and placed upon a glass slide under a covering glass, which is fixed to the slide with vaseline or melted paraffin. The main difference in the staining of the two sorts of leucocytes consists in the staining of granules, which, in the normal leucocytes, are beautifully stained red, and are present in such numbers as to render

the nucleus almost invisible. In the tuberculous leucocytes these granules have disappeared, and the nucleus is very distinctly seen, surrounded by unstained protoplasm. The difference can also be demonstrated by an addition of aqueous neutral-red to the leucocytes. In the normal leucocytes the granules stain red, whereas the tuberculous leucocytes have no granules. This difference is caused by a different reaction of the protoplasm in the leucocytes. The reaction is normally slightly acid. In the tuberculous leucocytes this acidity is diminished. This difference in the mode of vital staining is identical with that which is observed in the staining of living and dead leucocytes. If normal leucocytes and leucocytes which have been heated above the flame are mixed with Manson's stain quite the same differences will be seen. In living leucocytes the granules are stained, in heated leucocytes no granules will be seen. Leucocytes from the tuberculous animal must be considered as dead or dying and it must therefore be concluded that leucocytes from tuberculous organisms are more sensitive with regard to tuberculin than those from a normal organism. My experiments in this respect confirm the observation of Achard and Bénard (1909), who employed a technique which is unknown to me but state that "l'activité leucocytaire" in a solution of tuberculin is not so good in leucocytes from a tuberculous organism as in normal leucocytes.

The phenomenon which I have described above is constantly observed in the leucocytes of tuberculous guinea-pigs. I have been able to demonstrate it in eight out of nine tuberculous animals. Most striking were the changes where the tuberculosis was far advanced. Control animals (10) never showed the phenomenon, which must be regarded as a cellular allergic reaction, the tuberculin reaction of the cell.

SUMMARY.

In order to obtain knowledge of the fate of tuberculin in the organism the substance was injected into normal animals. After injection, tuberculin rapidly disappears from the vascular system and does not reappear in the urine till after several hours. In the meantime the substance is bound in the organism, probably in the bones and in the liver. Living cells *in vitro* also have the faculty of binding tuberculin.

Through numerous experiments a difference is demonstrated between the complements of different sera, a fact which strongly supports the theory of the plurality of complements. Evidence is brought forth which points to a difference between tuberculous and non-tuberculous complement.

Tuberculin is highly noxious to the vitality of the white blood corpuscles, and more toxic to corpuscles from tuberculous organisms than from non-tuberculous organisms. A certain protective power in regard to the toxic action of tuberculin is found in the serum. This power is greater in serum from normal organisms, than in serum from tuberculous organisms.

By means of vital staining a difference is demonstrated between the effects of tuberculin upon the leucocytes from normal and tuberculous animals.

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FURTHER RESULTS ON THE ISOLATION OF ORGANISMS FROM FAECES BY A NEW METHOD¹.

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IN a previous communication (Wordley, 1921) I fully described a method, introduced by Dudgeon, for isolating organisms from faeces and sputum. The material is dried to a powder and this dried powder spread over convenient culture media. From my observations this method, for faeces, was found to be superior to those generally in use for isolating the dysentery and enterica group of organisms. Further, this method is equally suitable whatever infection may be present and the procedure is the same in every case. Hitherto, different methods have been advocated for different infections, so that in cases in which the infection is doubtful or unknown, several different methods of isolating the causal organism might be necessary, while if only one method was used because of an erroneous preliminary diagnosis, a negative result would be obtained. Further, it was found that this dry method was most satisfactory for isolating faecal streptococci, especially if blood agar was used, for, using this medium, streptococci were isolated in every instance from any specimen of faeces, often in great abundance, and in addition a separation is obtained between haemolytic and non-haemolytic varieties of streptococci. Briefly the procedure is as follows. A portion of faeces about the size of a hazel nut is evenly spread over a sterile porous tile and allowed to partially dry; the material is then transferred to another tile and dried completely, so that it can be scraped off as a dry powder. This dry powder is then spread over a number of plates containing suitable culture media. Liquid stools and those containing much mucus can be dealt with just as easily in this manner. Further, during the process of drying, small pieces of mucus are shewn up and can be picked off for microscopy and the stool concentrated for protozoa, whereas they might have been missed on inspection of the whole stool. This method of drying on tiles will be found of great value in obtaining excellent separation of colonies from sputum, the drying does not appear to injure the most delicate organisms.

In my paper already referred to, the number of *B. typhosus* infections examined was limited, and since it has been impossible to secure any large number of stools from typhoid patients, recourse has been had, in the experiments detailed below, to artificial mixtures of faeces with *B. typhosus*. This organism was employed, as it is comparatively difficult to isolate, whereas *B. paratyphosus* B. is very readily isolated under all conditions. As an alter-

¹ The expenses of this investigation were defrayed by a grant from Mr Louis Oppenheimer.

native method, in order to provide a comparison, the brilliant green enrichment method of Browning (1919) was employed. A further comparison with the brilliant green method was advisable, as in my former paper the brilliant green tubes were only incubated for four hours before plating, instead of for 24 hours as recommended by Browning.

In all 100 stools have been examined by these two methods, the procedure adopted being as follows. Faeces were collected in small sterile pots from patients with no symptoms of intestinal disease. About a small teaspoonful of the sample was mixed with a sufficient quantity of sterile saline to make a thick emulsion. To this was added three drops of an emulsion of typhoid bacilli, prepared by adding 2 c.c. of saline to a 24 hours' growth of the organism on an agar slope. The mixture of faeces and typhoid bacilli was well mixed and one large loopful added to each of two brilliant green tubes. These tubes contained 5 c.c. of peptone water to which was added 0.1 c.c. and 0.2 c.c. of a 1/10,000 solution of the dye (Grübler's manufacture). The remainder of the typhoid-faeces mixture was spread on tiles and dried, and the resulting powder spread over plates. The peptone water brilliant green tubes were incubated at 37° C. for 24 hours and then plated, the plates incubated another 24 hours and any suspicious colonies picked off; similarly the plates inoculated with the dried powder were incubated at 37° C. for 24 hours, after which suspicious colonies were picked off and further tested. The media used for plating were MacConkey's lactose neutral-red bile salt agar, and litmus lactose agar. The results obtained are summarised in the following Table:

Table I.

No. of specimens examined	Positive results by dry method	Positive results by brilliant green
100	27	5

Among the 27 positive results obtained by the dry method are included two in which positive results were also obtained by the brilliant green method, while the five positive results from brilliant green include two examinations in which positive results were also obtained by the dry method. The results obtained in 100 examinations are also shown in Table II where a comparison is made between MacConkey's medium and litmus lactose agar.

Table II.

	No.
Positive results on litmus lactose agar only	19
Positive results on MacConkey's medium only	3
Positive results on both litmus lactose agar and MacConkey's medium	3
Positive results by brilliant green method	3
Positive results by brilliant green method and dry method	2
Total positive results	30

The brilliant green tubes were plated on both MacConkey's medium and litmus lactose agar, but the number of positive results is too small to separate.

It will be seen from the two Tables that the method of drying on tiles and plating direct gives much superior results compared with the brilliant green method; a similar conclusion was reached in my earlier paper (1921). Further, litmus lactose agar gives much better results than MacConkey's medium by this dry method. The one disadvantage of litmus lactose agar is that if the plate is heavily inoculated with faeces so much acidity is produced that all colonies appear red from a diffusion of the dye and colonies of *B. typhosus* have to be recognised by their characteristic naked eye appearance apart from any special colour of the colony, though where one is fairly constantly dealing with "enterica" infections, this presents less difficulty.

From constant use of the above method, it has been found superior to any of the methods commonly in use for isolating organisms from faeces. It effects a great saving in culture media since it is a method that is equally applicable to any infection or for the isolation of any organisms that may be present in faeces. Also it is equally serviceable for sputum, giving very much better separation of colonies than other methods and thus effecting much saving of time in obtaining pure cultures of individual organisms such as may be necessary in preparing vaccines for infections of the respiratory tract.

REFERENCES.

- BROWNING, C. H. (1919). *Applied Bacteriology*. Oxford Medical Publ. p. 95.
WORDLEY, E. (1921) A new method for the isolation of organisms from faeces and sputum, with some observations on Haemolytic streptococci in faeces obtained by this method. *Journ. Hygiene*, xx. p. 60.

PULMONARY TUBERCULOSIS AND THE "CURVATURE OF VAN PESCH."

BY C. EIJKMAN,

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(With 2 Text-figs.)

IN 1885, the Dutch vital statistician, Prof. Van Pesch¹ drew attention to a peculiarity of the curve of death rates at ages which he first noticed in the Netherlands' statistics of 1870-79 and subsequently again found in the three following decades.

This peculiarity, sometimes called the "curvature of Van Pesch," consists in an inflection of the generally concave arc especially prominent in the curve of death-rates of males at or about the 22nd year of life, less distinct and beginning one or two years earlier in the curve of female mortality which, owing perhaps to the incidence of diseases at the age of procreation, returns more gradually to the general trend. As will be seen from Fig. 1, this inflection is not peculiar to the Dutch statistics, but is present in greater or less degree in those of other nations².

I have ascertained that, at least in the statistics of the Netherlands, the curvature is due to mortality from phthisis.

Phthisis mortality statistics show two maxima, one between the ages of 20 and 30, the other between 50 and 65. The latter is most accentuated in urban populations, being in the curve of male mortality even absolutely greater than the young adult maximum; the curve conforms to Brownlee's Old Age Type³. In rural populations, the maximum for males at older ages is also found but is smaller than in urban populations, the curve is intermediate in type between Brownlee's old age form and his young adult type.

The course of mortality from phthisis amongst women conforms still more closely to the young adult type, especially in rural populations.

Undoubtedly in both sexes an increase of the mortality from phthisis between the ages of 10 and 30 exists and corresponds fairly well both in degree and position to the "curvature of Van Pesch." The latter disappears if we

¹ A. J. van Pesch (1885), *Bijdragen van het Statistisch Instituut*, No. 3. Haarlem.

² Its greater prominence in the male mortality curves of countries having compulsory military service has been emphasised by certain military writers, e.g. O. v. Schjerning (1910), *Sanitätsstatistische Betrachtungen ueber Volk und Heer*, Berlin: Hirschwald, pp. 86 *et seq.* See, however *Journ. Roy. Statistical Soc.* LXXIII. 253.

³ Brownlee, J. *Medical Research Council, Special Report Series*, No. 18 of 1918 and No. 46 of 1920.

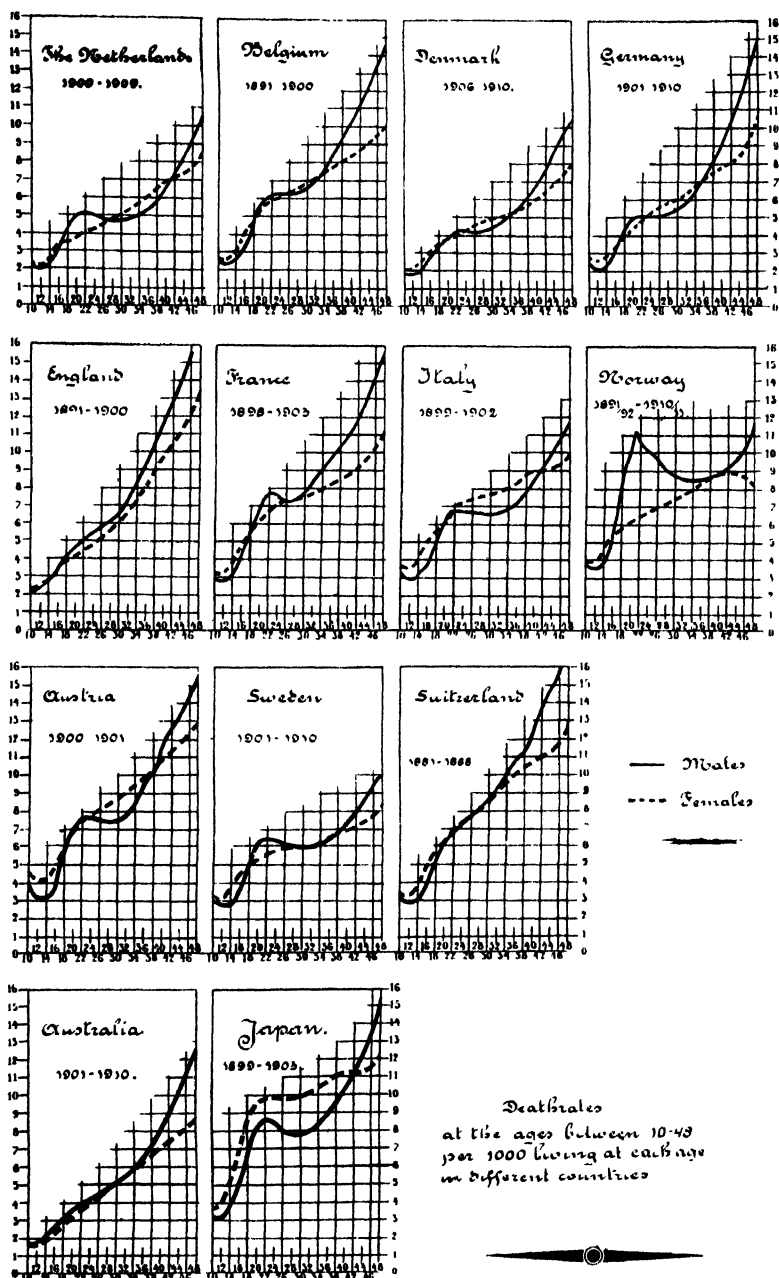


Fig. 1

subtract the phthisis death rates from the general death rates, as will be seen in Fig. 2 which illustrates the general mortality curve both inclusive and exclusive of phthisis for males in the age-grouping customary in Dutch statistics, viz. 5-13, 14-19, 20-29, 30-39, 40-49 and 50-64 years.

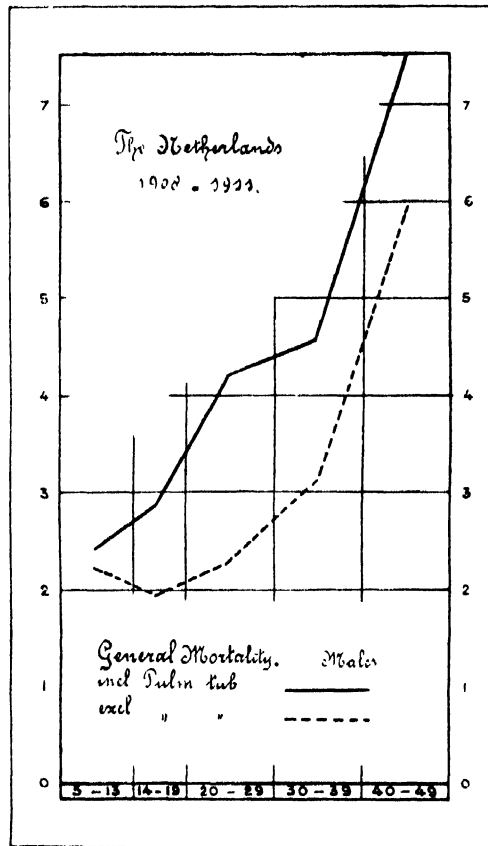


Fig. 2.

THE DIET IN THE DIFFERENT GRADES OF SOCIETY IN DENMARK.

BY DR POVL HEIBERG,
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IN the present communication a general survey of the diet in the different levels of society in Denmark will be given for the first time.

The material dealt with consists of about 1000 household accounts which the state statistical department has published in the course of time.

Table I

*Content per unit (or "man value") per day of protein, fat and carbohydrate,
and also calories in Danish diets.*

Year								Protein gm.	Fat gm.	Carbo- hydrate gm.	Calories
<i>Copenhagen.</i>											
1897	27	labouring	class	families	94	99	376	2848
1909	76	85	96	364	2730
1916	9	citizen families with income of 2000-3000 kroner						107	111	440	3276
..	49	3000-5000	..	105	113	452	3335
..	23	over 5000	..	118	114	475	3488
<i>Provincial towns.</i>											
1897	23	labouring	class	families	84	75	385	2617
1909	99	93	89	402	2863
1916	21	citizen families with income of 2000-3000 kroner						94	104	441	3166
..	41	3000-5000	..	91	103	439	3128
..	9	over 5000	..	103	98	522	3475
<i>Country districts.</i>											
1897	115	labouring class families, Islands			93	94	439	3056	
..	86	Jutland	102	89	473	3183
1909	65	104	104	512	3485
..	70	artisan families			105	105	491	3413
1916	15	citizen families with income of 2000- 3000 kroner						86	96	462	3142
..	37	3000-5000	..	91	107	459	3246
..	13	over 5000	..	104	121	480	3517
1909	84	peasant families			117	111	553	3778
..	142	farmer			149	132	650	4509

Table I shows the constitution of the diet per day and per unit, or "man value," for the whole of the material to hand. The units are relative figures which correspond to the normal food consumption for males and females, adults and children, as discovered by physiological experiments. The so-called American scale is the following:

Men over 15 years ...	1.00
Women over 15 years ...	0.90
Children from 11 to 14 years	0.90
„ „ 7 „ 10 „	0.75
„ „ 4 „ 6 „	0.40
„ under 4 years ...	0.15

Since in estimating the consumption in families of peasants and farmers some food may be included which in reality goes to domestic animals, these two groups of householders are not taken into account.

In Table I the figures for the daily amount of protein per unit lie between 84 and 118 gm. There is only one class—the most well-to-do Copenhagen families—which reaches the old standard of Voit of 118 gm. protein daily, but even the group which has the lowest daily consumption of protein, viz. 84 gm., may be said to get a sufficient amount of protein in the diet judging from our present knowledge of the subject.

For 12 of the 17 classes of families investigated the daily consumption per unit lies between 91 and 105 gm., only three being below and two above these limits. The figures for the daily consumption of fat per unit lie between 75 and 121, and the curve shows a well-defined apex about 100.

The amount of fat consumed therefore far exceeds the old standard of Voit—56 gm.—it is, in fact, more than double that quantity.

For 15 classes of families the amounts are between 89 and 114, and there is only one class above and one below these quantities.

The daily amount of carbohydrate per unit varies from 364 to 522 gm., the curve exhibiting a distinct apex at 450 gm. In 12 classes the daily consumption per unit lies between 402 and 480 gm., only three classes having a lower and two a higher consumption. These last two classes are the only ones which approach Voit's standard of 500 gm. per diem.

Table II shows the percentage of calories obtained from the proteins, fats and carbohydrates of the diet.

This table should render it obvious whether the various classes have chosen their diets economically. Since it is the proteins and fats that are more especially costly, it is most economical to draw upon the carbohydrates for a relatively large number of the calories required.

It will be clear from Table II how dear the mode of living is in all grades of society in Copenhagen in comparison with that in the provincial towns and country districts. Only the most well-to-do classes of the country families (for the most part doctor's families) live in the same costly manner as Copenhagen families.

About one-eighth of the calories are contributed by the proteins. The variations range from 11 to 14 per cent., in 13 cases however the values lie between 12 and 13½ per cent. The high figures apply particularly to Copenhagen. About three-tenths of the calories are derived from the fats. The variations range from 26 to 35 per cent., but the values have a marked

368 *Diet in Different Grades of Society in Denmark*

tendency to group themselves round 30 per cent. In this case also the high figures are most frequent in Copenhagen. But the percentage of calories supplied by the carbohydrates on the contrary are decidedly lower in Copenhagen than in either the provinces or the country districts. The variations range from 54 to 62 per cent., the curve showing a distinct apex at 58 per cent.

Table II.
*The percentage of calories in the diet contributed by
protein, fat and carbohydrate.*

Year							Protein gm.	Fat gm.	Carbo- hydrate gm.
<i>Copenhagen.</i>									
1897	27	labouring class families	13.5	32.3	54.1
1909	76	" " "	12.7	32.7	54.6
1916	9	citizen families with income of 2000-3000 kroner					13.4	31.5	55.1
"	49	" " " " 3000-5000	"				12.9	31.5	55.6
"	23	" " " " over 5000	"				13.9	30.4	55.8
<i>Provincial towns.</i>									
1897	23	labouring class families	13.1	26.6	60.2
1909	99	" " "	13.3	29.0	57.7
1916	21	citizen families with income of 2000-3000 kroner					12.2	30.6	57.2
"	41	" " " " 3000-5000	"				11.9	30.6	57.5
"	9	" " " " over 5000	"				12.2	26.2	61.6
<i>Country districts.</i>									
1897	115	labouring class families, Islands	12.5	28.6	58.9
"	86	" " " Jutland	13.1	26.0	60.9
1909	65	" " "	12.2	27.7	60.1
"	70	artisan families	12.6	28.6	58.9
1916	15	citizen families with income of 2000-3000 kroner					11.2	28.4	60.3
"	37	" " " " 3000-5000	"				11.5	30.6	57.9
"	13	" " " " over 5000	"				12.1	32.0	55.9
1909	84	peasant families	12.7	27.3	60.0
"	142	farmer	13.6	27.3	59.2

With regard to details of the diet of the various classes the reader is referred to the previously published articles both in Danish and German. An index of the most important of them is appended. In Table III, however, a condensed account of the whole material is given and also the coefficients necessary to convert the calculation of the number of calories for 1897 and 1909 from the Rübner scale used at that time to the so-called American scale in use in 1916.

In the years 1897 and 1909 the accounts of the families of labourers and artisans were registered. In 1909 the household accounts of peasants and farmers were added, and in 1916 those of public servants and the families of other citizens were obtained for the first time.

In 1897 an attempt was made in provincial towns to get as much insight as possible into the economic factors. The level of the wages of the families of the labouring class in that year lay only 10-15 per cent. above the mean

wages level, but in 1909 it was 30 per cent. above in the provincial towns. Both in Copenhagen where the accounts of the labouring classes in the two years named were respectively 60 and 45 per cent. above the average wages level, and in the provinces, the workers providing data were not a strictly random sample because the ability to produce accounts through a whole year that could be used in the investigation, stamped the labouring families concerned as being on a higher level.

Table III

General survey of the families investigated.

Year	No. of investigated families					No. of "American" units per family	No of individuals per family	Annual income per family in kroner	Coefficients for converting calories from the Rubner to the American scale	Expenditure on food expressed as a percentage of the total expenditure	
<i>Copenhagen.</i>											
1897	27	labouring class families				...	3.65	4.9	1706	0.85	47
1909	76	"	"	"	"	...	3.43	4.6	1740	0.84	47
1916	9	citizen families				...	2.76	—	2000-3000	—	36
"	49	"	"	"	"	...	3.00	—	3000-5000	—	30
"	23	"	"	"	"	...	3.82	—	over 5000	—	25
<i>Provincial towns.</i>											
1897	23	labouring class families				...	4.01	5.6	995	0.83	52
1909	99	"	"	"	"	...	3.47	5.0	1442	0.85	48
1916	21	citizen families				..	2.59	—	2000-3000	—	31
"	41	"	"	"	"	..	3.49	—	3000-5000	—	32
"	9	"	"	"	"	...	4.91	—	over 5000	—	25
<i>Country districts.</i>											
1897	115	labouring class families, Islands					3.46	4.9	781	0.85	57
"	86	"	"	"	"	Jutland	3.38	4.9	795	0.86	56
1909	65	"	"	"	"	...	3.32	4.7	924	0.87	64
"	70	artisan families				..	3.17	4.5	1052	0.88	55
1916	15	citizen				"	2.95	—	2000-3000	—	35
"	37	"	"	"	"	..	4.05	—	3000-5000	—	33
"	13	"	"	"	"	..	4.06	—	over 5000	—	25
1909	84	peasant				"	3.54	4.8*	1230	0.90	—
"	142	farmer				"	6.41	7.8†	2546	0.90	—

* 0.3 of which were engaged in the management of the household.

† 3.5 " " " " " " " "

In Copenhagen 1909 was a year marked by a good deal of unemployment.

Between 1897 and 1916 there was first of all a number of years of steadily increasing prosperity and steadily increasing level of prices for foodstuffs after which followed a couple of years with rapidly rising prices but without any increasing prosperity for the majority of families of ordinary citizens investigated in 1916, who for the most part are public servants (in the employ of the state or municipality).

From Table III it appears that the number of units in the case of the more

carefully examined 17 classes of families varied from 2.59 to 4.91 with the closest grouping around 3.50.

As the investigations were carried out in years with very different levels of prices a direct comparison of the yearly incomes is of little interest, but the relation between the expenditure on food and the total expenses of a family needs some attention. This relation ranged from 25 to 64 per cent. and, as is usual in such cases, it varied inversely as the income of the family.

The constants with which the number of calories and amounts consumed must be multiplied to transfer them from Rübner's scale to the American scale all lie between 0.83 and 0.88 for the labouring class families and artisans.

In spite of the wide scope of these Danish food investigations—1000 household accounts for a whole year—they cannot be looked upon as more than an indication.

It will fall to the lot of future research, profiting by the experience already gained, to confirm or modify the results here reported.

APPENDIX

The detailed physiological reports of the diet of the various classes of families will be found in the following articles.

In Danish.

1. The diet of the Danish labouring class families. *Maanedsskrift for Sundhedspleje*, 1910, p. 101.
2. The diet of the Danish labouring class families in 1897 and 1909. *Ibid.* 1913, pp. 145 and 214.
3. The diet of the Danish peasant families in 1909. *Ibid.* 1914, p. 263.
4. The diet of the Danish citizen families in the towns and country districts in 1916. *Ibid.* 1919, p. 105.

In German.

1. Die Kost dänischer Arbeiterfamilien. *Zentralbl. f. Stoffw. und Verdauungskrankheiten*, 1910, Nr. 12.
2. Die Kost dänischer Arbeiterfamilien in den Jahren 1897 und 1909. *Arch. für soziale Hyg. und Demographie*, 1914, p. 145.
3. Die Kost dänischer Bauernfamilien im Jahre 1909. *Arch. für soziale Hyg. und Demographie*, 1921, p. 257.

THE KEEPING QUALITIES OF GRADE A (CERTIFIED) MILK.

BY K. FREEAR, N.D.D., A. T. R. MATTICK, B.Sc.
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(With 1 Chart.)

IN two previous papers (Freear, Buckley and Stenhouse Williams, 1919; Freear, Mattick and Stenhouse Williams, 1921) accounts were given of the bacteriological condition of the milk from four Grade A (Certified) Farms, and it was shown that the milk from Farm 1 maintained a very high standard of bacteriological purity throughout a long series of examinations. In view of the fact that the keeping qualities of milk are of very great importance both to the industry and to the consumer, these have been studied with milk which was derived from Farm 1.

A paper concerning the keeping qualities of this milk, which was 24 hours old on arrival at the laboratory, has been published already (Freear, Buckley and Stenhouse Williams, 1919).

The present paper is concerned with the results which were found during the period August 18th, 1918-August 24th, 1919, when the milk was 30 hours old on arrival at the laboratory, and was morning's milk, whereas the milk previously discussed was evening's milk.

The milk was cooled at the farm, with water from a deep well, and the temperatures after cooling varied between a maximum of 62° F. and a minimum of 38° F. It was then run into pint bottles which were packed in ventilated boxes and dispatched by rail to the laboratory. No attempt was made to keep the milk cool during the journey with the result that it arrived at temperatures which varied between a maximum of 76° F. and a minimum of 38° F. The results of the study of the bacteriological condition of this milk have already been published, and it has been shown that its general cleanliness was of such a character that it is justifiable to consider its keeping qualities in relation to time and temperature conditions only, its bacteriological condition being regarded as constant (Freear, Mattick and Stenhouse Williams, 1921).

Condition of storage, and method of testing for sweetness.

In order to compare the effects of storage under different conditions, the milk was kept in four different places after its arrival at the laboratory; (1) in the ice chest, (2) in a fairly cool cellar; (3) on a slate slab four feet from

the floor of the laboratory; (4) on the laboratory floor beneath a window facing east, and eight feet from a gas fire. The experiments on the laboratory floor and slab were done in order to try and discover whether or not variations in the keeping qualities of milk could be demonstrated in different parts of the same room, and if so whether they would be of sufficient magnitude to be of practical value.

On its arrival the milk was thoroughly shaken, the caps were removed from the bottles, and samples for bacteriological tests were taken. Each sample was then divided into four parts by pouring through sterile funnels into sterile bottles which were then closed with plugs of sterile cotton wool. These plugs were removed twice daily for the purpose of tasting but no attempt was made to keep either the plugs or the mouths of the bottles sterile.

Table 1.

Average periods of sweetness of clean milk when stored in different places.

	Ice chest	Cellar	Slate	Floor
No. of samples	57	57	49	49
Average number of days sweet	14.25	5.7	4.64	5.02
Minimum number of days sweet	2	1.5	1.5	1.0
Maximum number of days sweet	25	15.5	11.5	11.5

Study of periods of sweetness.

Table I shows the periods of sweetness of the samples which have been reckoned from the time of milking. It demonstrates that the average period of sweetness of the samples which were kept in the ice chest was 14½ days

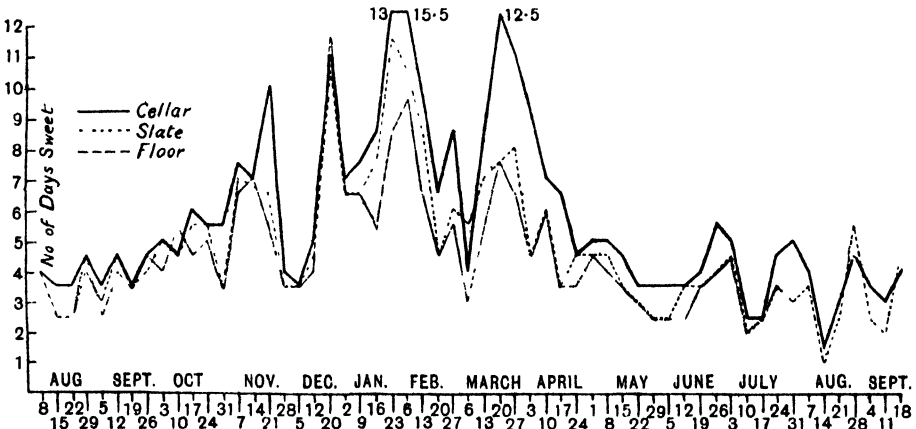


Chart 1. Showing the variation in keeping quality when samples of clean milk were divided into three parts and kept: (1) in a cellar, (2) on the floor of the laboratory, (3) on a slate slab in the laboratory 4 ft. above the floor.

with a minimum of two days, in the cellar 5.7 days with a minimum of 1.5 days, on the laboratory floor five days with a minimum of one day and on the slate in the laboratory 4.64 days with a minimum of 1.5 days. Chart 1 demonstrates the same facts.

It is clear that even under the very varying conditions of cooling, of carriage and of storage on arrival, this milk remained sweet for prolonged periods of time, since no sample was received in a sour condition and only one remained sweet for less than two days. It is interesting to note that samples of milk have been coming from this farm from November 1916 to the present time (Sept. 1921) and on no occasion has a sample been received in a sour condition. The chart further makes clear the phenomenon which is generally described as "seasonal influence," since it demonstrates the effects of temperature, cooling, transit and subsequent storage upon the keeping qualities of the milk.

In order that the facts contained in the chart may be more readily appreciated they have been epitomised in Table II which makes clear the marked influence of variations of temperature, etc. upon the keeping qualities of the milk at different seasons of the year. It will be seen that these were markedly less during the months May–September than they were during the months October–April.

Table II.

Table showing influence of temperature, etc. on keeping qualities at different periods of the year.

Period	Cellar		Floor*		Slate*	
	No.	Days sweet	No.	Days sweet	No.	Days sweet
May–Sept.	29	3.84	21	3.3	21	3.3
Oct.–April	28	7.62	28	6.22	28	5.6

* Floor and slate samples ceased on July 24th, 1919. Therefore, only 21 samples were examined during the summer period.

If it be granted that the bacteriological condition, and the cleanliness of the utensils used for this milk, were as good as are likely to be found in practice, which we believe to be true, then it is possible to consider other factors which make for sweetness or sourness in milk. Among these may be mentioned the temperatures of cooling and transit and the temperatures of subsequent storage.

The influences of temperatures of cooling and transit may be found from the results obtained with those samples which were subsequently kept in the ice chest, and the influences of the temperatures of storage, in those samples which were kept in the cellar and the laboratory.

Influence of temperatures of cooling and carriage.

The great importance of the temperatures of cooling and carriage on the subsequent keeping qualities of the milk, is shown in Table III.

From Table III it is seen that when the milk was kept in the ice chest those samples which had been cooled to an average temperature of 50° F. and arrived at temperatures between 41° F. and 50° F. remained sweet for an average period of 14.4 days, those which had been cooled to an average temperature of 55° F. and arrived at temperatures between 51°–60° F. re-

mained sweet for 14 days and those which had been cooled to an average temperature of 56° F. and arrived at temperatures which varied between 61°–70° F. remained sweet for 13.5 days. The temperatures of cooling and of carriage, therefore, had an appreciable influence upon the subsequent sweetness of these samples.

Table III.

Influence of temperature and age on arrival on the keeping qualities of samples of milk when stored in the ice chest.

Age in hours when tested	No. of samples	Average temperature of cooling	Average temperature on arrival	No. of days sweet
24	12	50° F.	41°–50° F.	{ 17.5
30	15			{ 14.4
24	14	55° F.	51°–60° F.	{ 16.8
30	17			{ 14.0
24	15	56° F.	61°–70° F.	{ 14.0
30	16			{ 13.5

It is of interest to consider the average periods during which the samples of milk remained sweet in the ice chest when they were 24 hours old on arrival, in comparison with the average periods when the milk was 30 hours old on arrival. This comparison has been made in Table III which shows that those samples which were 24 hours old on arrival, kept sweet in the ice chest for longer periods than those at similar temperatures which were 30 hours old on arrival and that this difference diminishes with the increase of temperature on arrival. Thus when the milk arrived at temperatures between 41°–50° F. the samples which were 24 hours old kept for an average period of 17.5 days, whereas those which were 30 hours old kept for an average period of 14.4 days—a difference of three days. When the temperatures on arrival were between 51°–60° F. the difference was 2.8 days. On the other hand when the milk arrived at temperatures between 61° F. and 70° F. the influence of increased temperature is shown by the fact that the difference was only half a day.

Influence of temperature on delivery and subsequent storage on the keeping qualities.

Table IV brings out the influence of the temperature on delivery and subsequent storage on the keeping qualities of the milk.

The samples which arrived at temperatures lower than 40° F. and above 70° F. are too few in number for discussion. They are included in order to complete the record.

It will be noted that those samples which arrived at temperatures between 61° F. and 70° F. could not be relied upon to keep sweet in the laboratory for a longer period than 2.8 days from the time of milking. At the present time the regulations permit of the sale of this milk when 48 hours old.

If we assume that the milk is 48 hours old when sold to the consumer then there is a margin of only 0.8 day sweetness in the consumer's house, a conclusion arrived at under conditions which were certainly not worse than the

Table IV.

The influence of temperature on arrival and subsequent storage on the period of sweetness of clean milk.

ICE CHEST										CELLAR				
No. of samples	Temp. in °F.	Age in hours	Temp. after cooling	Temp. on delivery	No. of days sweet	Average			No. of days sweet	Average				
						°F.	°F.	°F.		°F.	°F.	°F.	°F.	
1	40 or less	30	44	38	23	33	36	33	34.5	15.5	34	42	37	38.5
15	41 to 50	30.5	50	46	14.4	31	38	33.5	35	7.7	36	51	43.5	45
17	51 to 60	29.7	55	55	14.0	32	42	33.5	35.5	5.0	40	61	52	54
16	61 to 70	30	56	64.5	13.5	33	43	34	36.7	3.6	50	66	58	60
1	73	29	62	73	17	33	39	34	36.5	3.5	61	67	62	65
1	76	28	62	76	14	33	39	34	37	3.5	59	63	59.5	62
LABORATORY SLAB										LABORATORY FLOOR				
1	40 or less	30	44	38	9.5	33	62	39	51	10.5	31	53	36	46.5
15	41 to 50	30.5	50	46	5.5	39	71	47	55	6.2	34	57	44.5	51
17	51 to 60	29.7	55	55	4.25	42	72	53.5	59	4.5	39	70.5	51.9	58
16	61 to 70	30	56	64.5	2.8	59	73	61.5	69	3.0	53	76	60	68
1	73	29	62	73	2.5	67	78	67	78	2.5	63	79	62	79
1	76	28	62	76	2.5	57	71	57	71	2.5	57	71	57	71

average. Under the present regulations it does not appear advisable that milk of this age should be delivered to the consumer when it has been allowed to reach temperatures above 60° F.

If it were possible to deliver this milk within 24 hours of milking then the conditions would be altered and the matter would require reconsideration. It is a question for the industry to decide whether they will guarantee that the milk shall be cooled to a temperature of not more than 60° F. and be carried and stored at temperatures which shall not exceed this, with delivery at the end of 48 hours, or whether they will reduce the period of time during which the milk is in transit so as to guarantee its sweetness in the consumer's house. If the former course is taken then it will be necessary to adopt more efficient methods of cooling milk and keeping it cool during transit than exist at present.

If the latter, then the milk should be delivered to the consumer within 24 hours of milking if it is to be guaranteed to keep sweet for a further 24 hours.

The question whether more efficient methods of cooling during the summer months with delayed periods of delivery shall be adopted, or whether the present methods of cooling and transit, but with more rapid delivery, shall continue, is an economic problem which ought to be seriously considered by the industry, and upon which a decision should be reached.

Note.

Any decisions at which Grade A (Certified) Producers may arrive must take into account two fundamental facts. (1) That milk shall not be delivered to the consumer which will go sour before the next delivery. (2) That this milk is subject to official bacteriological examinations at any time before it reaches the consumer.

These two problems have been considered, the first in this paper and the second in the *Journal of Hygiene*, xx. No. 2.

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PLAGUE IN SOUTH AFRICA: PERPETUATION AND SPREAD OF INFECTION BY WILD RODENTS.

BY DR J. ALEXANDER MITCHELL,

Secretary for Public Health and Chief Health Officer for the Union.

(Read at South African Medical Congress, Cape Town, 9th October, 1921.)

THE infection of plague was originally introduced into South Africa during the Anglo-Boer War, 1900–1902, by rats from vessels with cargoes of forage from infected South American ports. During this period serious outbreaks occurred in Cape Town, Port Elizabeth, East London and other centres in the Cape Province, and also at Durban and Maritzburg. Further outbreaks—at Kingwilliamstown, Queenstown and elsewhere—occurred in 1903, and in 1904 a considerable outbreak occurred in Johannesburg and neighbourhood. The infection persisted at Port Elizabeth, East London and Johannesburg up to 1905.

In all these outbreaks the epidemic in man was associated with, and for the most part caused by, a plague epizootic amongst the local rodents. This was mainly confined to rats, both black and brown, and to ordinary domestic mice, but during the outbreaks at Knysna, Mossel Bay and Graaff Reinet, specimens of wild “striped mice” (*Arvicanthus pumilio*) were found dead of plague on the outskirts or in the vicinity of the infected towns. The mortality among these animals was most marked at Knysna. The forest and bush in the neighbourhood of the town teemed with these animals and an extensive plague epizootic occurred amongst them, infected specimens being found as far as 18 miles from the town.

During the period 1906–1911 no plague in man or animals was discovered in the Union, but in 1912 an outbreak occurred at Durban, comprising 32 cases with 26 deaths, concurrently with a plague epizootic amongst rodents in the dock area and in three or four localities in the town. The precise source of infection was not traced, but there is no reason to doubt that it was introduced by infected rats from vessels from eastern ports.

In 1914 a very virulent outbreak, mainly of pneumonic type, occurred amongst persons living on a remote farm in the Tarka district of the Cape Province. None of the persons first attacked had recently been away from the farm and no clue to the source of infection could be discovered. Considerable spread took place—the neighbouring districts of Queenstown, Middelburg and Glen Grey being affected, and later, the Uitenhage district. In these districts in 1914 there were 35 cases with 31 deaths; in 1915, 45 cases with 26 deaths; in 1916, 24 cases with 13 deaths; and in 1917, 2 cases—both fatal. All the

foregoing cases occurred on farms and in native locations in the rural parts of the districts where ordinary domestic rodents were very few or entirely absent; no trace of infection of wild rodents or other animals could be discovered. No case of plague is known to have occurred in the Cape Province since 1917.

In 1916 cases of plague began to occur on scattered farms in the Hoopstad, Winburg and Senekal districts of the Free State, totalling during the year 37 cases with 23 deaths. This prevalence continued during 1917, when 29 cases with 21 deaths occurred in the same districts, with an extension to the neighbouring Transvaal district of Potchefstroom, where 15 cases—14 of which were fatal—occurred. During 1918, 2 cases, both fatal, occurred in the Hoopstad district. In none of these outbreaks could the original source of infection be traced. The late Dr D. C. Rees who was for a time, jointly with Dr Targett Adams, in charge of plague work in the Free State emphasised the danger of mild ambulatory cases, and in a report dated 25th April, 1916, cited instances where the *bacillus pestis* in virulent form was present in the sputum of pure "bubonic" cases for as long as two months after recovery. But in many of these outbreaks no evidence of human conveyance could be traced, and in some the circumstances were such as to render this mode of conveyance highly improbable; also, it would be difficult to account for the persistence of infection in these districts over several years if the disease affected the human population only.

After the two cases in the Hoopstad district in September, 1918, there was a period of 18 months during which nothing suspicious occurred, and it was hoped that the infection had disappeared. But in March, 1920, an outbreak occurred amongst natives on the farm "Angra Pequena" in the Bothaville area of the Kroonstad district, 25–30 miles distant from farms in the Hoopstad district where cases occurred 18 months to two years previously. Despite careful investigation, the source of infection remained a complete mystery. During the last two months of 1920 several cases occurred on two farms in the Hoopstad district—near farms which had been infected two or three years before. Since the beginning of the present year, seven further small outbreaks have occurred in the Bothaville area of the Kroonstad district and the northern part of the Hoopstad district adjoining.

Careful inspections and investigations last year failed to disclose any evidence of plague amongst rodents. The ordinary species of domestic rodent are practically non-existent in the rural areas of these districts. It was felt, however, that there was strong presumptive evidence of some undiscovered agency in the persistence and spread of the infection. The Departmental records and reports of the outbreaks in the Free State from 1916 onwards were carefully scrutinised and reviewed by Dr Haydon and myself, and the seats of the outbreaks plotted on a large scale map. Subsequently, a general inspection of the area was made by Dr Haydon. Arrangements were made with the authorities of the Pretoria Museum for a rodent survey of the area

to be carried out by Mr Austin Roberts, Naturalist to the Museum, with the assistance of Mr Powell, an expert tracker and trapper. A valuable report on the rodent and small carnivora populations of the area was furnished by Mr Roberts. During the course of his investigations several decomposed or dried-up carcasses of wild rodents were found, and the fresher of them submitted to the South African Institute for Medical Research, Johannesburg, for examination, but with negative results. It was found that the gerbille or "nacht-muis" (*Gerbillus taterona*) was very numerous in many parts of the area, also the multimammate mouse (*Rattus coucha*), the large-eared mouse (*Malacothrix typicus*), and the striped mouse (*Arvicanthus pumilio*), together with the yellow mongoose (*Cynictus penicillata*), the ground squirrel (*Geosciurus capensis*), and the suricat or true meercat.

On going further into the matter we found that the distribution of outbreaks corresponded roughly with the areas or sandy stretches in which gerbilles were especially prevalent, and other circumstances came to light concentrating special suspicion on these animals. Evidences of recent migration of gerbilles and desertion of burrows were also found, and in a few instances dried-up or mummified carcasses or skeletons of dead gerbilles. Owing to the activities of the small carnivora, birds and ants, the carcasses of animals dying in the open generally disappear very quickly.

Search parties under the direction of Mr Powell, the expert tracker and trapper, with Dr Sheldon and, later, Dr Levisseur, as Plague Medical Officers in local charge, were organised by the Department. A large number of burrows in likely places were dug out and arrangements made for using healthy wild rodents as bait, by placing them in cages near suspected burrows in the hope that they would exchange fleas with the inmates of the latter, but for long the results were negative.

The habits of the gerbille are purely nocturnal, so that they are rarely seen by man; also, they rarely or never enter dwellings. They are gregarious and migratory and often travel long distances at night, either singly or in parties. The almost complete destruction of jackals, lynxes and cats and the great development of mealie-growing have created conditions exceptionally favourable to wild rodents in this part of the Free State. The place of the ordinary domestic mouse is taken by the multimammate mouse, which lives sometimes in gerbille burrows and sometimes in dwellings or outbuildings, or, again, it may alternate between the two. It is a lazy animal, never travels far, and prefers any cover, or the hole or burrow of some other animal, to digging a hole for itself.

It was further found that these and other veld rodents, and the small carnivora living in association with them, were flea-infested, especially during the summer time. A collection of fleas and other ecto-parasites of these animals was made; the species have since been identified and include a number of fleas which bite man (see list annexed, for which I am indebted to Mr G. A. H. Bedford, Entomologist, Veterinary Research Institute, Onderstepoort, Pre-

toria). Healthy specimens of these animals tested at the South African Institute for Medical Research, Johannesburg, were found to be highly susceptible to plague. In the bulletin issued by the Department for the week ended 4 December, 1920, it was stated:

The general circumstances of the recurring small outbreaks of Plague since 1916 in the north-western part of the Orange Free State and neighbouring part of the Transvaal suggest that the infection exists amongst and is being perpetuated and spread by wild rodents; the gerbille or nachtmuis, the large-eared mouse (*Malacothrix*) and the veld muis (*Multimammate Mouse*) are especially suspect. Notwithstanding careful search and investigation, however, no direct evidence of this has so far been found. The investigations and precautions are being continued.

In February last the owner of the farm "Grootdraai," close to Bothaville, and about eight miles from "Angra Pequina," became ill and died of plague. Around his homestead were a number of gerbille burrows, but the inmates of these were healthy. For a week or 10 days before the onset of his illness the farmer had been ploughing on lands some three miles from the dwelling and was wont to have a mid-day siesta under the shade of a clump of bush near by. This locality was searched and several recently dead gerbilles and multimammate mice were found on the ground surface; there was also a large colony of burrows. These were excavated and a total of eight dead gerbilles found—the remainder having evidently migrated. There were also found between 350 and 400 multimammate mice, of which 150 were recently dead and 100 were obviously sick; the remainder were kept in captivity and most of them died during the succeeding week. Specimens of the dead gerbilles and multimammate mice and also of the mice which died in captivity later on were sent to the South African Institute for Medical Research, Johannesburg, and found to be plague-infected. Thus ended successfully a long and tedious investigation.

Since then, plague-infected gerbilles and multimammate mice have been found on another farm, "Dwaalfontein," in the Bothaville district, on which cases of plague had occurred—also on the farm "Cato's Vlei" in the Hoopstad district. Dried-up or decomposed carcasses of gerbilles, multimammate mice and malacothrix have been found on several other farms in the area, but all were useless for bacteriological examination.

So far the only species in which plague infection has been definitely found are gerbilles and multimammate mice. Two dried-up carcasses of ground squirrels have been found on a farm on which plague-infected gerbilles were discovered. As yet no definite evidence incriminating the small carnivora, such as the mungoose and muishond, has been found.

There is little doubt that plague infection has existed amongst the wild rodents in the north-western part of the Free State since 1916, the gerbille being probably the main factor in its persistence and spread, the multimammate mouse being in many cases the connecting link by which infected fleas were conveyed to man.

The area at present involved is, roughly, that bounded on the north by the Valsch River, on the west by the Vaal River, on the east by the main railway line, and on the south by the Vet River with a strip of infected country on its lower side—a total area of about 5000 square miles.

The elucidation of the chief mode of persistence and spread of the infection is an important step in the direction of limitation and eradication, but the problem is still a very difficult one and the risks of spread to the wild rodents in neighbouring districts and to the domestic rodents in the towns and villages, or its conveyance by rail or otherwise to some of the large towns, are very great. Ordinary methods of poisoning and trapping are quite useless for dealing with these animals; gassing with carbon bisulphide is the most effective means we have yet been able to devise.

No plague in man or rodents has been discovered since April last, but with the return of warm weather and the increase of insect life a recrudescence of the disease may be expected.

Plague is known to have occurred and persisted in enzootic form amongst wild rodents in other countries. Some years ago in California the disease was proved to exist amongst ground squirrels (*Citellus Beecheyi*), and the original source of infection of the terrible Manchurian epidemic of pneumonic plague of the winter of 1910–1911—in which some 50,000 cases occurred without a single recovery—is believed to have been the Tarbagan or marmot (*arctomys*). I understand that certain species of gerbille are common in India and it seems possible that our investigations in South Africa may throw light on some of the plague problems of other countries.

The original source of infection of the wild rodents in the north-western Free State must remain a matter of surmise. Natives from Tarka, Queens-town and neighbouring districts of the Cape Province sometimes go to work on the farms in the Free State, and the infection may have been introduced in 1916 in this way and conveyed from man to rodent by fleas. On the other hand, however, it seems well within the bounds of possibility that the infection has existed amongst wild rodents in certain parts of the Union since the outbreaks of 1903, and that the outbreaks in the Tarka and other midland districts of the Cape Province in 1914–1917 were caused in the same way. At first sight one would expect that had the infection so persisted, outbreaks would have been more frequent and continuous, but it must be borne in mind that the complicated chain of infection between rodent, flea and man can, in the nature of things, rarely be completed.

*Plague in South Africa**List of Fleas collected from wild rodents and small carnivora in North-Western Free State.*

ANIMAL HOST	FLEAS
<i>Tatera lobengulae</i> (Gerbille)	<i>Dinopsyllus lypsus</i> <i>Xenopsylla eridos</i> <i>Listropsylla stygius</i>
<i>Rattus coucha</i> (Multimammate Mouse)	<i>Dinopsyllus lypsus</i> <i>Echidnophaga larina</i>
<i>Rattus rattus</i>	<i>Xenopsylla cheopis</i>
<i>R. rattus fruginis</i>	<i>Xenopsylla brasiliensis</i>
<i>Arvicanthus pumilio</i> (Striped Mouse)	<i>Dinopsyllus lypsus</i> <i>Xenopsylla eridos</i> <i>Chastopsylla octava</i> <i>Listropsylla stygius</i>
<i>Laggada</i> sp.	<i>Listropsylla stygius</i>
<i>Malacothrix</i> sp.	(<i>Dinopsyllus lypsus</i> (<i>Listropsylla stygius</i>
<i>Steatomys</i> sp.	<i>Listropsylla stygius</i>
<i>Xerus (Geosciurus) capensis</i> (Ground Squirrel)	<i>Xenopsylla isidis</i> <i>Echidnophaga bradyta</i> <i>Echidnophaga gallinaceus</i> <i>Ctenocephalus canis</i>
<i>Cynictus penicillata</i> (Yellow Mongoose)	<i>Echidnophaga bradyta</i> <i>Xenopsylla isidis</i> <i>Echidnophaga gallinaceus</i> <i>Ctenocephalus canis</i> <i>Listropsylla stygius</i>
<i>Pedetes caffer</i> (Spring Hare)	<i>Xenopsylla</i> nov. sp.
<i>Suricator suricator</i> (Suricat or True Meercat)	<i>Ctenocephalus canis</i> <i>Echidnophaga gallinaceus</i>

THE RELIABILITY OF THE WASSERMANN TEST AS PERFORMED BY DIFFERENT PATHOLOGISTS.

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THE object of the work here recorded was to discover in how far the results of Wassermann tests, performed by pathologists working under the various Venereal Diseases Treatment Schemes of the Local Government Board in Ireland, agreed with one another. The authorities of five pathological laboratories, in which 88 per cent. of all the Wassermann tests done under the schemes last year were performed, kindly consented to co-operate in this experiment. These pathologists, to whom my best thanks are due for their help and interest, are subsequently described as *A*, *B*, *C*, *D*, and *E*, in order to preserve their anonymity.

Samples of blood were obtained from thirty patients, attending Venereal Diseases Treatment Centres. To the medical officers of these centres—at Sir Patrick Dun's Hospital and Dr Steeven's Hospital, Dublin, the Royal Victoria Hospital and the Mater Infirmorum Hospital, Belfast—my thanks are due for their kindness in obtaining for me the necessary blood and also for the clinical details of the cases.

In Table I the cases are arranged in six groups, in accordance with their clinical condition.

Table I.

Group	Clinical diagnosis	Numbers
1	Primary syphilis (undergoing treatment)	2, 4, 7, 8, 12, 20, 24, 26, 27
2	Secondary syphilis (untreated)	1, 22
3	Secondary syphilis (undergoing treatment)	3, 6, 9, 10, 13, 14, 15, 16, 17, 18, 19, 21, 25, 28, 29, 30
4	Syphilis with involvement of central nervous system (undergoing treatment)	5
5	Clinically doubtful (untreated)	23
6	Clinically not syphilis (untreated)	11

The patients were all males. All treated cases had received injections of Novarsenobillon intravenously and, in the majority of the cases, mercury cream intramuscularly.

The samples of blood were taken in my presence and handed over to me. I separated the serum and divided it into five parts, one of which was sent to each pathologist, under a number of my own. In no case, therefore, did

the pathologist know anything of the patient from whom the serum came. Four batches of sera were sent by post to each of the five pathologists. Batch 1 consisted of the sera of patients 1 to 7, batch 2 patients 8 to 14, batch 3 patients 15 to 23 and batch 4 patients 24 to 30. An interval of one or more weeks elapsed between the collection of each batch of bloods. The pathologists reported their results to me as soon as their examinations of each batch had been made.

Before giving the results the method of performing the Wassermann test employed by the collaborators will be indicated.

Pathologist A.

The method is that described by Harrison (method No. 1, Medical Research Committee's Report, No. 14, 1918).

The results are recorded thus:

++
+±
+
±
±
—

Pathologist B.

The method is that of Harrison, except that the antigen is one prepared by Messrs Burroughs, Wellcome and Co. It consists of human heart extract and cholesterol in one solution. The results are recorded in the same way as those of pathologist A.

Pathologist C.

Harrison's method is slightly modified by C. The antigen used is a bullock heart extract containing 0.4 per cent. of cholesterol. It is used in a dilution of about 1/30, the exact dilution being determined by titrations of its power of inhibiting the action of complement in the presence of and in the absence of a positive Wassermann serum. The tubes contain the following reagents:

		TUBE 1	TUBE 2	TUBE 3 (control)
Patient's serum	...	0.1 c.c.	0.1 c.c.	0.2 c.c.
Antigen (1/30)	...	0.5	0.5	—
Saline	—	—	0.5
Complement (3 M.H.D.)	...	1.0	—	1.0
Complement (5 M.H.D.)	...	—	1.0	—

Fixation, 1 hour in water bath at 37° C.

Sensitised cells	...	1.0	1.0	1.0
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The results are recorded thus:

++
< ++
+
±
+

Pathologist D.

The method is that of McIntosh and Fildes (*Syphilis from the Modern Standpoint*, 1911). This is a "one tube" method. That is, there is only one tube containing patient's serum, complement and antigen. A second tube containing serum and complement acts as a "serum control." The antigen is an alcoholic extract of human heart, without cholesterol. (McIntosh and Fildes now employ an antigen which contains cholesterol.) The tubes contain:

	TUBE 1	TUBE 2 (control)
Saline	0.74 c.c.	0.8 c.c.
Antigen (undilute) ...	0.06	—
Patient's serum ...	0.1	0.1
Complement (2½ M.H.D.)	0.1	0.1
Fixation, 1 hour at 37° C.		
Sensitised cells ...	0.5	0.5

The necessary controls for antigen, complement, etc., are also set up. The results are recorded thus:

+4
+3
+2
+1
Negative

Pathologist E.

This is a two tube method, two antigens being used.

Antigen 1. Alcoholic extract of human heart. Diluted 1/25.

Antigen 2. The same with 0.4 per cent. cholesterol added. The tubes contain:

	TUBE 1	TUBE 2	TUBE 3 (control)
Patient's serum ...	0.1 c.c.	0.1 c.c.	0.2 c.c.
Complement (2 M.H.D.)	0.5	0.5	0.5
Antigen 1 (1/25) ...	0.5	—	—
Antigen 2 (1/25) ...	—	0.5	—
Saline	—	—	0.5
Fixation, 1 hour at 37° C.			
Sensitised cells ...	0.5	0.5	0.5

The results are recorded in the same way as those of *D*.

Owing to the different notations in use by these five pathologists, it became necessary to reduce them to a common expression. This was accomplished

Table II.

Suggested symbol	Interpretation	A and B	C	D	E
<i>N</i>	Negative	—	—	—	—
<i>NP</i>	Some inhibition. In- sufficient for diagnosis but useful in control- ling treatment	±, ±	±, ±	+1, +2	+1
<i>P</i>	Definitely positive	+	+	+3	+2
<i>PP</i>	More strongly positive	+±, ++	< ++, ++	+4	+3, +4

by using letters as symbols. Table II shows the individual symbols used by each worker and those adopted in the new method.

The next table (III) gives the results of the tests all reduced to the new system. In the last column the majority result is stated.

Table III.

Serum	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	Majority result
1	<i>PP</i>	<i>NP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
2	<i>PP</i>	<i>N</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
3	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>PP</i>	<i>PP</i>
4	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>
5	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
6	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>N</i>	<i>P</i>	<i>P - PP</i>
7	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>NP</i>	<i>N</i>
8	<i>NP</i>	<i>PP</i>	<i>N</i>	<i>NP</i>	<i>PP</i>	(?) ¹
9	<i>P</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>NP</i>	<i>N</i>
10	<i>PP</i>	<i>PP</i>	<i>NP</i>	<i>NP</i>	<i>P</i>	<i>P - PP</i>
11	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>
12	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>P</i>	<i>PP</i>	<i>PP</i>
13	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>PP</i>	<i>PP</i>
14	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>NP</i>	<i>N</i>
15	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>PP</i>	<i>PP</i>
16	<i>NP</i>	<i>N</i>	<i>NP</i>	<i>NP</i>	<i>P</i>	<i>NP</i>
17	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
18	<i>PP</i>	<i>P</i>	<i>P</i>	<i>N</i>	<i>NP</i>	<i>P - PP</i>
19	<i>PP</i>	<i>P</i>	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>PP</i>
20	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>
21	<i>PP</i>	<i>N</i>	<i>P</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
22	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
23	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
24	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>
25	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
26	<i>NP</i>	<i>N</i>	<i>NP</i>	<i>N</i>	<i>NP</i>	<i>NP</i>
27	<i>N</i>	<i>N</i>	<i>N</i>	<i>N</i>	<i>NP</i>	<i>N</i>
28	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>PP</i>
29	<i>N</i>	<i>P</i>	<i>NP</i>	<i>N</i>	<i>NP</i>	<i>N - NP</i>
30	<i>PP</i>	<i>PP</i>	<i>PP</i>	<i>P</i>	<i>PP</i>	<i>PP</i>

¹ It has been found impossible, owing to the wide variations recorded by the pathologists, to state the majority result for this serum. There is, in general, a tendency towards the positive side.

It is now possible to compare the results of the five pathologists. In the case of ten sera (33·33 per cent.) there is absolute agreement between the five. In six of these the result is *PP* and in four it is *N*. In the case of six more of the sera (20 per cent.) the only difference is between *P* and *PP*. Since this is not of great importance clinically it may be taken that in these six results the pathologists are also agreed. So there is agreement between all the pathologists as to the results being positive or negative in 53·33 per cent. of the cases. In the results of seven sera (23·33 per cent.) four agree with one another and the fifth differs. Three agree as to the results while two differ from the majority in the case of five sera (16·66 per cent.). Only in the case of two sera is there lack of agreement between any three pathologists.

We may now consider the differences between these five pathologists' returns. Variations between *P* and *PP* are disregarded.,

Table IV.

Pathologist	Serum No.	Report of pathologist	Majority report
<i>A</i>	9	<i>P</i>	<i>N</i>
<i>B</i>	1	<i>NP</i>	<i>PP</i>
	2	<i>N</i>	<i>PP</i>
	16	<i>N</i>	<i>NP</i>
	21	<i>N</i>	<i>PP</i>
	26	<i>N</i>	<i>NP</i>
	29	<i>P</i>	<i>N - NP</i>
<i>C</i>	8	<i>N</i>	<i>NP - PP</i>
	10	<i>NP</i>	<i>P - PP</i>
<i>D</i>	6	<i>N</i>	<i>P - PP</i>
	10	<i>NP</i>	<i>P - PP</i>
	18	<i>N</i>	<i>P - PP</i>
	26	<i>N</i>	<i>NP</i>
<i>E</i>	7	<i>NP</i>	<i>N</i>
	9	<i>NP</i>	<i>N</i>
	14	<i>NP</i>	<i>N</i>
	16	<i>P</i>	<i>NP</i>
	18	<i>NP</i>	<i>P - PP</i>
	27	<i>NP</i>	<i>N</i>

It should be noted that five of *B*'s six divergences from the majority lie in swinging the result to the negative side and that *D*'s four are of exactly the same nature. *E*, on the other hand, makes the results of five sera more positive than they really are. It is probable that the antigens used by *B* and *D* are not sufficiently sensitive, while that of *E* is too sensitive for the amount of complement employed.

The errors may be divided into two classes, the serious and the moderate. In the first group are those in which the report is *N* while the majority are *P* or *PP*, and those in which the report is *P* or *PP* while the majority report is *N*. Such errors may cause the clinician to make mistakes either in the diagnosis or treatment of his cases.

Less serious errors are those of degree, *N* for *NP* and vice versa, *P* or *PP* for *NP* and *NP* for *P* or *PP*. These errors may deceive the clinician, but are less likely to do so than those in the first group.

Working on this basis, the results of each pathologist may be compared. The results have been tabulated in Table V.

Table V.

	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Number of results agreeing with majority	29	24	28	26	24
Percentage of results which agree with majority (assumed to be correct)	96.66	80	93.33	86.66	80
Serious errors	1	2	1	2	0
Percentage of serious errors ...	3.33	6.66	3.33	6.66	0
Moderate errors	0	4	1	2	6
Percentage of moderate errors	0	13.33	3.33	6.66	20

The next question to consider is the reliability of each pathologist's method. There is no doubt that the first place goes to *A* and the second to *C*. There is also no doubt that *B* deserves the last place. The question whether *D* or *E* should be placed third is more difficult. *D* has only made four errors as against *E*'s six, but two of *D*'s are serious, while four of *E*'s errors are very slight, consisting in reporting negative as *NP* (in one, "a trace to 1"; in the second, "a trace"; in the third, " + 1"; and in the fourth, "a trace only"). It is difficult to decide between *D* and *E* but, on the whole, despite *E*'s greater number of errors, I am inclined to put *E*'s results as somewhat more reliable than *D*'s, chiefly on the ground of absence of gross errors in *E*'s results. The order of reliability is, therefore,

A
C
E
D
B

Considerable attention has been paid to the question of errors, but one must not fail to observe that even the least reliable pathologist has given results which agree in 80 per cent. of the specimens examined with those of the majority, which are taken to be the correct results.

In 15 sera the correct result was clearly strongly positive (*PP*). In all 75 tests of these 15 sera were made by the five pathologists and in only three cases (4 per cent.) were the results not definitely positive. These three errors were made by one pathologist (*B*). On the eight clearly negative sera, 40 tests were made and in five reports the results were not clear negatives. Of these five errors, four were made by one pathologist and consisted in reporting the results as *NP* ("a trace," etc.).

Errors were made chiefly in the case of sera which were weakly positive, that is, border-line cases in which it is always difficult to obtain uniform results.

One hundred and fifty examinations in all were done and correct results were returned in 131, that is, in 87 per cent. The serious errors only amounted to 4 per cent. of all examinations.

It may, therefore, be claimed that, in performing the Wassermann test, correct results are obtained in a greater percentage of the cases than in practically any other clinical test in existence.

Although the results of the control experiment here recorded are satisfactory, it would be desirable, if possible, to secure even greater uniformity. In order to do so, the following recommendations are made:

1. A uniform method should be adopted by all the pathologists working under the schemes of the Local Government Board in Ireland. My preference would be for that of Harrison which is now used by three of the pathologists.
2. The details of the method should be rigidly standardised, especially as regards the following: antigen, haemolysin, blood suspension, time and temperature of fixation.

As regards the first two, it would appear well to supply to each pathologist a uniform, tested antigen, and a carefully tested haemolytic serum. A supply could be issued every month or two, and so it could be made certain that the same batch was in use by each pathologist at the same time. In the report of the Medical Research Committee already referred to, this was recommended, but no steps have been taken to secure such a supply. It would be possible for the Board to undertake the manufacture and supply of these two articles. As regards the suspensions of blood cells used, I believe it to be of great importance to secure suspensions of uniform strength. A simple method of doing so has been described by me elsewhere¹.

3. A uniform method of reporting the results of the test should be adopted.

CONCLUSIONS.

A considerable degree of uniformity of results of the Wassermann test has been found among five pathologists.

Certain differences (chiefly in the case of weakly positive sera) have, however, been noted. Their main source lies in the use of different methods and not in any lack of personal ability or care on the part of the worker.

It is believed that the number of these could be greatly reduced by adopting a uniform method of performing the test and by standardising the details of the method.

The supply of a standard antigen and haemolysin to pathologists is recommended.

¹ Bigger. "The Standardisation of suspensions of Red Blood Cells for Wassermann Tests." *Lancet*. 1921, ii. 1369.

INDEX OF AUTHORS.

	PAGE
BIGGER, JOSEPH W. The Reliability of the Wassermann Test as performed by different Pathologists	383
BRUCE, Sir D. Trench Fever. Final Report of the War Office Trench Fever Committee	258
BUXTON, J. B., and ALLEN, H. R. Air carriage of pathogenic and other organisms	173
DUDGEON, L. S., WORDLEY, E., and BAWTREE, F. On <i>Bacillus coli</i> Infection of the Urinary Tract, especially in Relation to Haemolytic Organisms	137
ELJKMAN, C. Pulmonary Tuberculosis and the "Curvature of van Pesch." (With 2 Text-figures)	363
FREEAR, K., MATTICK, A. T. R., and WILLIAMS, R. STENHOUSE. A Study of the Bacteriological Examination of Grade "A" (Cert.) Milk	125
FREEAR, K., MATTICK, A. T. R., and WILLIAMS, R. STENHOUSE. The Keeping Qualities of Grade "A" (Cert.) Milk. (With 1 Chart)	371
GALE, A. M. Some Points in the Epidemiology of an Outbreak of Cerebro-Spinal Fever in Hong-Kong, 1918. (With 3 Charts, 2 Maps, and 1 Figure)	289
GARDNER, A. D. and WALKER, E. W. AINLEY. An Inquiry into the Nature of the Serological Differences exhibited by different Cultures of a Bacterial Species (<i>B. typhosus</i>). (With 5 Charts)	110
GLENNY, A. T., and SÜDMERSEN, A. J. Notes on the Production of Immunity to Diphtheria Toxin. (With 15 Charts)	176
HARRIES, E. H. R., and WILLIAMS, R. STENHOUSE. Bovine Tuberculosis: the Etiological Support of Family History	132
HEIBERG, POVL. The Diet in the Different Grades of Society in Denmark	366
HOLST, PETER M. Studies on the Effects of Tuberculin. (With 2 Text-figures)	342
KHALED, Z. A Comparative Study of Bovine Abortion and Undulant Fever, from the Bacteriological point of view. (With 2 Charts)	319
MATTICK, A. T. R. The Sterilization of Empty Milk Churns by Steam under Pressure	165
MITCHELL, J. ALEXANDER. Plague in South Africa: Perpetuation and Spread of Infection by Wild Rodents	377
PETRIE, G. F. The Toxigenic Features of Strains of the Diphtheria Bacillus isolated from Horses and from a Mule	99
SAVAGE, W. G. Studies upon the Toxicity of Putrid Food	69
SCHUTZE, H. The Permanence of the Serological Paratyphoid B Types, with observations on the Non-specificity of Agglutination with "Rough" Variants. (With 1 Figure)	330
TOPLEY, W. C. C. The Spread of Bacterial Infection. The Potential Infectivity of a surviving Mouse Population, and their Resistance to subsequent Epidemics of the same Disease. (With 2 Charts)	103
TOPLEY, W. C. C., BARNARD, J. E., and WILSON, G. S. A New Method of obtaining Cultures from Single Bacterial Cells. (With 1 Plate)	221
TOPLEY, W. C. C., WEIR, H. B., and WILSON, G. S. The Inter-relationships between the various members of the <i>B. enteritidis</i> - <i>B. paratyphosus</i> B Group of Bacteria. (With 3 Charts)	227
TWOOT, F. W., and TWOOT, D. N. An Investigation on Influenza	85
WILSON, W. H. The Diet Factor in Pellagra	1
WORDLEY, E. A New Method for the Isolation of Organisms from Faeces and Sputum, with some Observations on Haemolytic Streptococci in Faeces obtained by this Method	60
WORDLEY, E. Further Results on the Isolation of Organisms from Faeces by a New Method	360
YOUNG, M. A Preliminary Study of the Epidemiology of Rheumatic Fever	248

INDEX OF SUBJECTS.

	PAGE
Agglutination of <i>B. coli</i>	158
of <i>B. paratyphosus</i> B	330
Air carriage of organisms	173
<i>Bacillus abortus</i>	319
<i>coli</i> , agglutinins for	158
in urinary tract	137
<i>diphtheriae</i> , from horses	99
<i>enteritidis-paratyphosus</i> group	227
<i>influenzae</i>	85
<i>melitensis</i>	319
<i>paramelitensis</i>	319
<i>paratyphosus</i> B	330
<i>proteus</i> in putrid food	69
<i>typhosus</i> , serological differences in cultures of	110
Bacteria, cultures from single cells	221
Bacterial infection, spread of	103
Bovine abortion	319
tuberculosis	132
Cerebro-spinal fever	289
Cultures from single organisms	221
Diet in Denmark	366
Diphtheria toxin, immunity to	176
Epidemiology of cerebro-spinal fever	289
rheumatic fever	248
Faeces, tile method of isolating organisms from	60, 360
Fever, cerebro-spinal	289
trench	258
Food, putrid, toxicity of	69
Horses, <i>B. diphtheriae</i> from	99
Infection, spread of	103
Influenza	85
Immunity to diphtheria toxin, production of	176
Milk, bacteriology of Grade "A"	125
keeping qualities of Grade "A"	371
Milk-churns, sterilization of	165
Pellagra	I
Plague in South Africa, spread of	377
Rheumatic fever, epidemiology of	248
Rodents and plague in South Africa	377

	PAGE
Serological differences of cultures of <i>B. typhosus</i>	110
paratyphoid B types	330
Sputum, isolation of organisms from	60
<i>Streptococci</i> , haemolytic, from faeces	64
Tiles for isolating organisms from faeces	60, 360
Tuberculin, effects of	342
Tuberculosis, bovine	132
and "curvature of van Pesch"	363
Trench fever	258
Urinary tract, <i>B. coli</i> infections	137
Wassermann test, reliability of	383

I. A. R. I. 75

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